Tolerion, Inc.

Diabete**S** A**U**toimmu**N**ity Withd**R**awn **I**n New On**SE**t and In E**s**tablish**e**d Patients

(SUNRISE)

A Phase 2 Multi-Center, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety and Efficacy of TOL-3021 in Patients with New Onset or Established Type 1 Diabetes Mellitus

Protocol No. TOL-3021-231		
Phase 2		
Version	**Version 1**	**Date:** 20 February 2019
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Sponsor:	**Tolerion, Inc.** **131 Oyster Point Blvd, Suite 400** **South San Francisco, CA 94080**	

Clinical Development Approval:

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Executive Vice-President
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Date

PROTOCOL / AMENDMENT APPROVAL PAGES(S)

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Investigator Agreement Page

I have carefully read Protocol TOL-3021-231 entitled: "A Phase 2 Multi-Center, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety and Efficacy of TOL-3021 in Patients with New Onset or Established Type 1 Diabetes Mellitus" and,

I agree that it contains all the necessary information for me and my personnel to conduct this study as described.

I will provide copies of the protocol, any subsequent protocol amendments, and access to all information provided by the Sponsor to the study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the investigational drug and the study protocol.

I agree to conduct this clinical trial according to the attached protocol, except when mutually agreed to in writing. I also agree to conduct this study in compliance with all federal, state, and local regulations, as well as with the requirements of the appropriate Institutional Review Board (IRB) / Ethics Committee (EC) and any other institutional requirements.

Principal Investigator Signature	Date (ddmmmyyyy)

Printed Name:
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STUDY SYNOPSIS

Study Title	DiabeteS AUtoimmuNity WithdRawn In New OnSEt and In Established Patients (SUNRISE) A Phase 2 Multi-Center, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety and Efficacy of TOL-3021 in Patients with New Onset or Established Type 1 Diabetes Mellitus

Protocol Number	TOL-3021-231
Sponsor	Tolerion, Inc.
Name of Finished Product	TOL-3021, 1.0 mg
Active Ingredient	A plasmid expression vector containing the coding sequences for full-length human proinsulin.
Comparator Product	Placebo
Objectives	**Primary Efficacy Objective** To evaluate the effect of TOL-3021 dosing over 24 weeks on preserving endogenous insulin secretion as reflected by C-peptide secretion, and its effect on other measures of efficacy in patients with Type 1 Diabetes Mellitus (T1D). **Primary Safety Objective** To evaluate the safety of TOL-3021 administered as weekly intramuscular (IM) injections over 52 weeks in patients with Type 1 Diabetes Mellitus (T1D).
Study Description	The study is a prospective, randomized, 52-week, placebo-controlled, multicenter trial in subjects with T1D followed by a 2-year safety follow-up. The trial is triple-blinded through the analysis at Week 24, after which the Sponsor will be group unblinded. The study will continue through Week 52 as double-blinded with site staff and subjects blinded.
Study Population	Up to 99 male or female subjects aged 12 to 40 years (Cohort A) with a minimum of 18 and a maximum of 24 subjects aged 12-<18 (Cohort B) diagnosed with T1D as defined by the American Diabetes Association (ADA) criteria and meeting enrollment criteria as specified below may be enrolled.

Eligibility Criteria	**Inclusion Criteria** 1. Diagnosis of Type 1 Diabetes Mellitus based on ADA criteria and within 5.0 years from diagnosis, defined as the first day of insulin administration. 2. Age at randomization of 12.0 – <41.0 years of age[1]. 3. Adequate glycemic control as defined by HbA1c ≤7.9% based on point-of-care or local lab measurement and time in glycemic range (70-180 mg/dL) >55% by CGM recording over 3 or more consecutive or non-consecutive days within 5 days prior to baseline mixed meal tolerance test (MMTT). 4. On insulin therapy (total insulin dose >0.125 U/kg BW) 5. Presence of antibodies to at least one of the following antigens: GAD65, IA-2, ZnT8, or insulin if obtained within 10 days of the onset of exogenous insulin therapy, or documentation of positive antibodies. In the absence of a positive result for one of the specified antibodies, diagnosis of T1D as per the ADA guidelines. 6. Peak C-peptide during screening 4-hour mixed meal tolerance test (MMTT) ≥ 0.150 nmol/L. 7. Willingness to wear the Dexcom G6 continuous glucose monitoring (CGM) device and use according to instructions including recording of total daily insulin dose taken most of each day from screening to end of treatment period. 8. Written informed consent and for subjects aged 12-<18 years of age, subject assent and parental or guardian consent, including authorization to release health information. 9. Willingness and ability of subject to comply with all study procedures of the study protocol, including attending all clinic visits. **Exclusion Criteria** 1. Receiving a dose of acetaminophen >4,000 mg per day. 2. Body Mass Index (BMI) >32 kg/m^2 for patients 18 and older (>85th percentile for ages 12-17). 3. Previous immunotherapy for T1D within 2 years of enrollment. 4. Diagnosis of liver disease or hepatic enzymes, as defined by ALT and/or AST ≥ 2.5 times the upper limit of normal (ULN). 5. Hematology: white blood cells (WBC) <3 x 10^9/L; platelets <100 x 10^9/L; hemoglobin <10.0 g/dL. (Low WBC values may be repeated every 3-7 days, and results to be discussed with the Medical

[1] A window is provided for qualifying of within +90 days from the 41.0-year cutoff.

	Monitor.) Any underlying conditions likely to impact red blood cell turnover. 6. Latent autoimmune diabetes of adults (LADA), which is generally associated with preceding history and treatment of T2D with medications typically used for treatment of T2D for more than 30 days. 7. Monogenic diabetes (MODY). 8. Estimated glomerular filtration rate (eGFR) <60 ml/min for ages >18-<41, and <75 ml/min per 1.73 m^2 for ages 12-<18. 9. History of malignancy, except for cancers in remission >5 years, or basal cell or *in situ* squamous cell carcinoma of the skin. 10. Significant cardiovascular disease (including inadequately controlled hypertension), history of myocardial infarction, unstable angina, use of anti-anginal medicines (e.g., nitroglycerin), or abnormal stress test, which, in the opinion of the Principal Investigator (PI), would interfere with participation in the trial. 11. Immunosuppressive therapy (systemic corticosteroids, cyclosporine, azathioprine, or biologics) within 30 days of screening. 12. Current or prior (within the last 30 days) use of metformin, sulfonylureas, glinides, thiazolidinediones, GLP1-RAs, DPP-IV inhibitors, pramlintide, or SGLT-2 inhibitors. 13. Current use of verapamil or α-methyldopa. 14. History of any organ transplant, including islet cell transplant. 15. Asthma that requires oral glucocorticoid therapy. Inhaled glucocorticoid therapy is permitted. 16. Active autoimmune or immune deficiency disorder including rheumatoid arthritis, moderate-to-severe psoriasis, inflammatory bowel disease, and other autoimmune conditions that may require treatment with TNF or other biologics. Permitted autoimmune disorders include T1D or well-controlled autoimmune conditions (e.g., thyroid disease, celiac disease, and sarcoidosis, all with stable non-immunosuppressive medications for the past 30 days). 17. Thyroid-stimulating hormone (TSH) at screening >7.5 mIU/L for ages 18-<41 years old and > 3.6mIU/L for ages 12-<18 years old. 18. Adrenal insufficiency that is not adequately treated with stable replacement glucocorticoid therapy. 19. Moderate non-proliferative retinopathy (NPDR) or proliferative retinopathy

	20. Evidence of infection with HBV (as defined by hepatitis B surface antigen, HBsAg), HCV (anti-HCV antibodies), or HIV. 21. Subject is breastfeeding. 22. Positive urine pregnancy test at screening or at any time during the study (pregnancy tests must be performed as per the visit schedule) : Females of childbearing potential must be excluded if they have a positive urine pregnancy test at screening or randomization or if they are not using medically acceptable methods of birth control. Acceptable methods of birth control include oral or transdermal contraceptives, condom, spermicidal foam, IUD, progestin implant or injection, abstinence, vaginal ring, or sterilization of partner. The reason for non-childbearing potential, such as bilateral tubal ligation, bilateral oophorectomy, hysterectomy, or 1 year or more postmenopausal must be specified in the subject's Case Report Form (CRF). 23. Males of reproductive potential who are unwilling to use medically acceptable birth control, unless the female partner is postmenopausal or surgically sterile. 24. Any social condition or medical condition that would, in the opinion of the PI, prevent complete participation in the study or would pose a significant hazard to the subject's participation. 25. Anticipated major surgery during the duration of the trial, which could interfere with participation in the trial. 26. History of drug or alcohol dependence within 12 months of screening. 27. Psychiatric disorder that would prevent subjects from giving informed consent. 28. Household members of current participants in this protocol. 29. Subjects who are not fluent in the English language. 30. Participation in other studies involving the administration of an investigational drug or experimental device, including the administration of an experimental agent for T1D within 30 days of screening, or use of an experimental therapeutic device for T1D within 30 days prior to screening. Subjects previously treated with diagnostic devices are not excluded. 31. Any current use of biotin or biotin containing supplements.
Study Drug Administration	TOL-3021 1.0 mg or placebo will be administered weekly for 52 weeks via an intramuscular (IM) injection into a large muscle.
Study Procedures	Up to 99 subjects aged 12.0 to <41.0 years (Cohort A), including a minimum of 18 and a maximum of 24 subjects aged 12-<18 (Cohort

	B), will be randomized in a 2:1 ratio to receive active or placebo study drug. Patients aged 18-<41.0 years will considered Cohort C. Subjects will qualify after meeting all study enrollment criteria and will be randomized within 5.0 years of confirmed T1D diagnosis, defined as the first day of insulin administration. Subjects will be stratified by T1D duration (zero up to 1 year, which defines new onset disease and 1 year up to five years, which defines established T1D) to ensure balance of disease duration across treatment and placebo groups in each stratum. Subjects should be randomized no sooner than 6 weeks after diagnosis, unless glycemic range is adequately controlled as confirmed by time in glycemic range (70-180 mg/dL) >55% by CGM recording over 3 or more consecutive or non-consecutive days. The CGM record should be taken within 5 days prior to the screening (baseline) MMTT. Subjects will receive study drug as an IM injection into a large muscle each week for 52 weeks. Initial treatments will be administered at the clinical site at randomization and at the Week 1 and Week 2 clinic visits. During those visits, subjects and/or a caregiver will be trained to administer study drug at home in subsequent weeks. Study drug will be dispensed at the Week 2 visit for Week 3 administration at home, except for the first 6 subjects aged 14-<18 years of age in Cohort B. These subjects will have a visit at Week 3 and will receive their injection at the site. Week 4 study drug will be administered at the clinic visit. Safety assessments will be conducted at each visit and study drug for at home use will be dispensed at Weeks 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48. An additional safety assessment visit will occur at Week 54. For subjects aged 12-<18 (Cohort B), dosing will be staggered with the initial 6 subjects aged 14-<18 enrolled with the last subject having a minimum of 2 injections with at least 1 week follow-up after the 2nd injection. Safety data from these initial subjects will be evaluated before opening the study to subjects 12 and older. A 4-hour MMTT will be conducted during Screening, and at Week 12, Week 16, Week 24, and Week 52. The screening result will be used as the baseline value. CGM will be initiated 5 or more days before the Screening MMTT visit and will continue throughout the 52-week treatment period. The MMTT may be conducted as part of a single day screening visit if the subject is using a CGM device that permits sharing of data with site staff prior to the visit so that time-in-range blood glucose values can be assessed prior to the MMTT. Safety assessments and additional efficacy assessments will be conducted at each visit (see APPENDIX 1, Schedule of Assessments). Subjects will record information in a diary to capture symptomatic hypoglycemia events and study drug administration during the study. Daily insulin use and glucose levels will be captured using the CGM device.

	A Data Safety Monitoring Board (DSMB) was established prior to study initiation to review data throughout the study, including the Week 12 data review described below. When the first 30 treated subjects completed the Week 12 visit, all available data was compiled and provided to the DSMB to consider if the enrollment of adolescent subjects (aged 12 - <18) in TOL-3021 studies can be recommended. Summary and individual subject data were provided for DSMB and FDA review. If no objection is received from regulators and IRBs, then sites will be notified that they may begin enrollment of subjects aged 12 - < 18. In addition, an interim analysis will be performed when the first 30 subjects have completed 24 weeks of treatment to evaluate the treatment response. Alpha spending will be performed using an O'Brien-Fleming approach. Subjects will be asked to continue in the study for a 2-year safety follow-up period after completion of the 52-week double-blind study. During this 2-year follow-up subjects will have clinic visits at 18, 24, and 36 months from their first dose in the blinded study to evaluate safety. At each of these follow-up visits, a MMTT will be administered.

Study Outcomes	**Primary Efficacy Outcome**
	The primary outcome is the TOL-3021 treatment effect as determined by a repeated measures analysis of change from baseline in the log-transformed MMTT C-peptide AUC at 12, 16, and 24 weeks.
	
	Secondary Efficacy Outcomes
	
	Secondary outcomes will include the treatment effect on:
	
	• Rates of clinically important hypoglycemia events as defined by total measured glucose value of <54 mg/dL (3.0 mM/L) over each approximately 12-week period ending at Weeks 12, 24, 36, and 52:
	  o A single blood glucose level.
	  o By CGM, ≥10 consecutive minutes with glucose <54 mg/dL
	• Total daily insulin requirements in units per kilogram (kg) body weight.
	• HbA1c.
	• Time in glycemic range 70 - 180 mg/dL
	
	Other Secondary Endpoints
	The TOL-3021 treatment effect—
	• On a repeated measures analysis of change from baseline in the log-transformed MMTT C-peptide AUC at 12, 16, 24, and 52 weeks.

	• On number of times the CGM reports glucose levels of <70 and <55 mg/dL • On a clinical responder analysis defined as no change or an increase in C-peptide AUC from baseline between treatment and placebo at Weeks 12, 16, and 24 weeks. Upon completion of 52 week data, a similar analysis will include the 52 week data. • On non-fasting or fasting C-peptide (single test) from baseline at Weeks 12, 16, 24, and 52. • On proportion of subjects in each treatment arm with HbA1c levels less than 6.5% at Week 52. • On CGM parameters: o Time >180 mg/dL o Time >250 mg/dL o Mean Glucose Coefficient of Variation o Low Blood Glucose Index (LBGI) o Glucose below 70 mg/dL o Area Over the Curve (AOC_{70}) • On other measures of hypoglycemia: • Severe hypoglycemia (SH) events (impaired or loss of consciousness requiring assistance of another). o Documented symptomatic hypoglycemia (an event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration <70 mg/dl (3.9 mmol/L)). Total time <70 mg/dL by CGM. o Nocturnal hypoglycemia, i.e., severe or documented symptomatic episodes (as defined above) occurring after the subject has retired for the primary sleeping period. <u>Immunologic</u>: • Quantum dot (Q-dot) responses within the qualifying subpopulation to confirm induction of specific autoantigen tolerance. • Comparison of quantum dot responses within the qualifying subpopulation to clinical outcomes to confirm correlation with specific autoantigen tolerance. • Determine effect of treatment on and predictive value of: o Regulatory/protective humoral immune response to proinsulin/insulin; o Serum insulin autoantibody affinity for subjects o Insulin autoantibody isotypes (IgA and IgM) and IgG subclasses;

	○ Serum insulin, glutamic acid decarboxylase, IA-2, and ZnT8 antibodies by radio-binding assay (RBA) assay; ○ Competition assays of serum insulin and proinsulin IgM and IgG antibodies. **Safety Outcomes** Safety variables include: • Clinical laboratory tests (hematology, chemistry, urinalysis); • Urine pregnancy test (UPT) for women of childbearing potential (WOCBP); • Concomitant medications • Collection of adverse events (AEs) • Vital signs • Injection site reactions • Severe hypoglycemia or hyperglycemia events monitored by CGM • Events of special interest: ○ Systemic or hypersensitivity reactions associated with injection, which consist of fever, chills, headache, nausea, vomiting, and/or other signs and symptoms, such as anaphylaxis, wheezing dyspnea, urticaria, and hypotension
Sample size	Up to 99 subjects (cohort A) including a minimum of 18 and a maximum of 24 subjects aged 12-<18 (cohort B) will be randomized 2:1 to active and placebo treatments. Patients aged 18-<40 will be considered Cohort C. The proposed study is powered to detect approximately an overall 13% difference between treatment and placebo groups in a repeated measures analysis of the change from baseline in log-transformed "mean" plasma C-peptide AUC over 4-hour MMTT at Weeks 12, 16, and 24. A total of 90 subjects will provide 90% power to detect this difference at a significance level (alpha) of 0.05; a total of up to 99 subjects will be randomized to allow for an anticipated drop-out rate of approximately 10%.
Data Handling and Blinding	Data will be collected and managed by The Emmes Company, LLC (Emmes) the Contract Research Organization (CRO). The data will be inspected for inconsistencies by performing validation checks and any inconsistencies found will be resolved by staff from Emmes (data management, clinical research associates, etc.) after contacting the investigator. Subjects and investigators will be blinded to treatment assignment throughout the study. Selected Emmes staff will have access to treatment assignments. An interim analysis will be performed after the first 30 subjects have completed the week 24 visit. Unblinded

	results will be provided to the DSMB. Primary analysis of the data will be conducted after all randomized participants have completed 24 weeks Cohorts B and C may be analyzed separately. Unblinded data summaries including safety outcomes and exploratory efficacy assessments will be prepared for the sponsor during the study using all data collected through Weeks 24 and 36.
Pharmacokinetic considerations	N/A
Statistical Analyses	**Analysis Populations** *Safety* The safety population will consist of all subjects who receive one or more doses of TOL-3021 or placebo and have any follow-up safety data. *Efficacy* The efficacy population is by intention-to-treat: all subjects randomized to treatment and taking at least one dose of medication will be included in the ITT population. Appropriate imputation methods may be used for missing data. **Statistical Methods** **Primary Efficacy Analysis** The analysis of the primary outcome, change from baseline in log-transformed MMTT-stimulated 4-hour mean C-peptide AUC at multiple time points will test the null hypothesis of "no treatment group difference" versus the two-sided alternative using the intent-to-treat (ITT) cohort. The primary hypothesis test will be the treatment group estimate from a repeated measures mixed model fit to a log-transformed 4-hour C-peptide AUC response at 12, 16 and 24 weeks, adjusted for the baseline C-peptide AUC, age group and duration group.
Estimated Trial Initiation Date	10 June 2019
Estimated 52-Week Trial Completion Date	30 Sep 2021
Estimated Follow-up Period Completion Date	30 Sep 2023

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or Term	Definition/Explanation
ADA	American Diabetes Association
AE	Adverse Event
ALT (SGPT)	Alanine aminotransferase (Serum Glutamic Pyruvic Transaminase)
ANA	Anti-nuclear antibody
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
Anti-dsDNA	Anti-double-stranded DNA
AST (SGOT)	Aspartate aminotransferase (Serum Glutamic Oxaloacetic Transaminase)
AUC	Area Under the Curve
BHT	Bayhill Therapeutics
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
BW	Body weight
C	Celsius
CBC	Complete Blood Count
CGM	Continuous Glucose Monitoring
CMV	Cytomegalovirus
CRA	Clinical Research Associate
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CRF	Case Report Form
CRO	Contract Research Organization
DMT	Diabetes Management Team
DPT	Diabetes Prevention Trial
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
ECL	Electrochemiluminescence
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GAD65	Glutamic acid decarboxylase
GCP	Good Clinical Practice
HbA1c	Glycosylated hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
hINS	Human proinsulin
HIV	Human immunodeficiency virus
HIPAA	Health Information Portability and Accountability Act of 1996

Abbreviation or Term	Definition/Explanation
HLA	Human leukocyte antigen
IA-2	Tyrosine phosphatase-like insulinoma antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent to Treat
IND	Investigational New Drug
IUD	Intrauterine Device
IV	Intravenous
Kg	Kilogram
LTFU	Long Term Follow-Up
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
mITT	Modified Intent to Treat
mL	Milliliter
MMTT	Mixed-Meal Tolerance Test
MOP	Manual of Procedures
NCI CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
Ng	Nanogram
PE	Physical examination
PI	Principal Investigator
RBA	Radio-binding assay
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SH	Severe hypoglycemia
SMBG	Self-monitored blood glucose
SOP	Standard Operating Procedures
SUNRISE	Diabete**S** A**U**toimmu**N**ity Withd**R**awn **I**n New On**SE**t and In E**s**tablish**e**d Patients
SUSAR	Serious Suspected Adverse Reaction
T1D	Type 1 Diabetes Mellitus
TEAE	Treatment Emergent Adverse Event
TOL	Tolerion
TNF	Tissue necrosis factor
TSH	Thyroid-stimulating hormone

Abbreviation or Term	Definition/Explanation
ULN	Upper limit of normal
UPT	Urine pregnancy test
US	United States
WBC	White Blood Cell
WOCBP	Women of childbearing potential
ZnT8	Zinc transporter 8

1 INTRODUCTION

Type 1A diabetes mellitus (T1D) is primarily a disease of children and young adults that results from immune-mediated destruction of the insulin-producing β-cells of the pancreas. In the United States, as many as 1.5 million individuals have T1D. Approximately one out of every 400–600 children and adolescents has T1D, with the incidence rates increasing in many geographical regions. Although clinical appearance of symptoms is often rapid, the decline in pancreatic function is gradual. Studies of individuals who are at high risk for developing diabetes have revealed that many years, or even decades, separate the first appearance of antibodies to pancreatic antigens and the destruction of enough β-cells to cause clinically apparent T1D (Yu et al. 2001).

The now widely accepted conclusion that T1D is an autoimmune disease has been based on several kinds of evidence. First, autopsy examination of T1D patients revealed a lymphocytic infiltrate in the pancreatic islets. Further examination of the infiltrate showed it to be composed predominantly of $CD8^+$ T cells and to be accompanied by up-regulation of HLA class I molecules within the islets (Roep 2003). This suggested a causative role for cytotoxic T lymphocytes, and further work has shown $CD4^+$ T cells to also be implicated in the autoimmune disease process, with a strong association of T1D with certain HLA class II haplotypes (Roep 2003). Finally, autoantibodies to pancreatic islet antigens have been found in the overwhelming majority of T1D patients and those at genetic risk for the disease who eventually develop diabetes. Autoantibodies to glutamic acid decarboxylase (GAD65), tyrosine phosphatase-like insulinoma antigen (IA2), and insulin are the most prevalent, with 95% of pre-diabetic or new-onset T1D patients positive for one or more of these antibodies, 80% positive for two or more, and 25% positive for all three. In contrast, only 3% of healthy controls are positive for one or more of these autoantibodies (Bonifacio et al. 1995). A large body of evidence suggests that insulin is a major, if not the predominant, autoantigen responsible for T1D pathogenesis.

Insulin is the only known β cell-specific autoantigen, and insulin autoantibodies are usually the first to appear in young children with T1D. All high-affinity insulin autoantibodies are reactive with proinsulin (Achenbach et al. 2004).

Furthermore, it was recently determined that 50% of the T cells isolated from pancreatic draining lymph nodes of patients with T1D recognize an epitope of the insulin A chain, whereas no T cells from healthy subjects recognize this epitope (Kent et al. 2005). Finally, it has been demonstrated that insulin-reactive T cells from T1D patients exhibit more of a destructive Th1 phenotype, whereas insulin-reactive T cells from healthy controls exhibit a protective T regulatory phenotype (Arif et al. 2004). Thus, a substantial rationale has developed for intervention against the autoimmune response against insulin in T1D patients.

Reducing autoimmune attack of β-cells is warranted, even for patients who have already presented the clinical disease. Although the presence of clinically detectable hyperglycemia signals that a significant percentage of β-cells have already been destroyed, almost all T1D patients have some residual insulin secretion at the time of diagnosis. For this reason, some patients experience a brief "honeymoon" period after initial clinical presentation, during which they do not require exogenous insulin or require it only at times of physiological stress. Residual insulin secretion declines post-diagnosis but is detectable for several years in most patients. For T1D patients who require insulin therapy, residual β-cell function is associated with improved short-term and long-term outcomes. For example, the contribution of residual β-cell function to glucose homeostasis appears to be associated with less glycemic variability (Palmer 2009, Greenbaum et al. 2012).

Although intensive management of exogenous insulin therapy to achieve strict control of hyperglycemia has been shown to slow the development of long-term complications, many patients inevitably and gradually develop complications, including damage to eyes, kidneys, and the cardiovascular system, which result in early disability and death. Such intensive management of insulin therapy also puts patients at higher risk for hypoglycemic events (DCCT 1993). It has been shown, on the other hand, that patients who retain substantial residual β-cell function have less hyperglycemia and hypoglycemia and have fewer long-term complications. The observation that the retention of pancreatic β-cell function improves short-term and long-term clinical outcomes is the rationale for attempts to ameliorate ongoing autoimmune destruction of β-cells. Given the immunological etiology of the disease, the most logical approach to preserving β-cell mass is to alter the immunological response that causes the disease.

In the Diabetes Prevention Trial, investigators administered insulin orally and by injection to individuals at high-risk for T1D in an effort to prevent diabetes development (DPT 2002). Unfortunately, this strategy did not work. A strategy that has shown more success is the use of non-specific immunosuppressive agents, particularly for the treatment of new onset diabetes. However, these approaches are often associated with transient but significant side effects including fever after the start of infusions, acute mononucleosis-like syndrome, and conversion to Epstein-Barr virus positivity by the end of treatment (Keymeulen et al. 2005).

TOL-3021, in contrast, is designed to modulate immune responses to insulin specifically to have no off-target immune-toxicity. This antigen-specific approach has the advantage of decreasing the autoimmune response while leaving other processes, such as immune surveillance against malignancy and immune responses against infectious agents, intact.

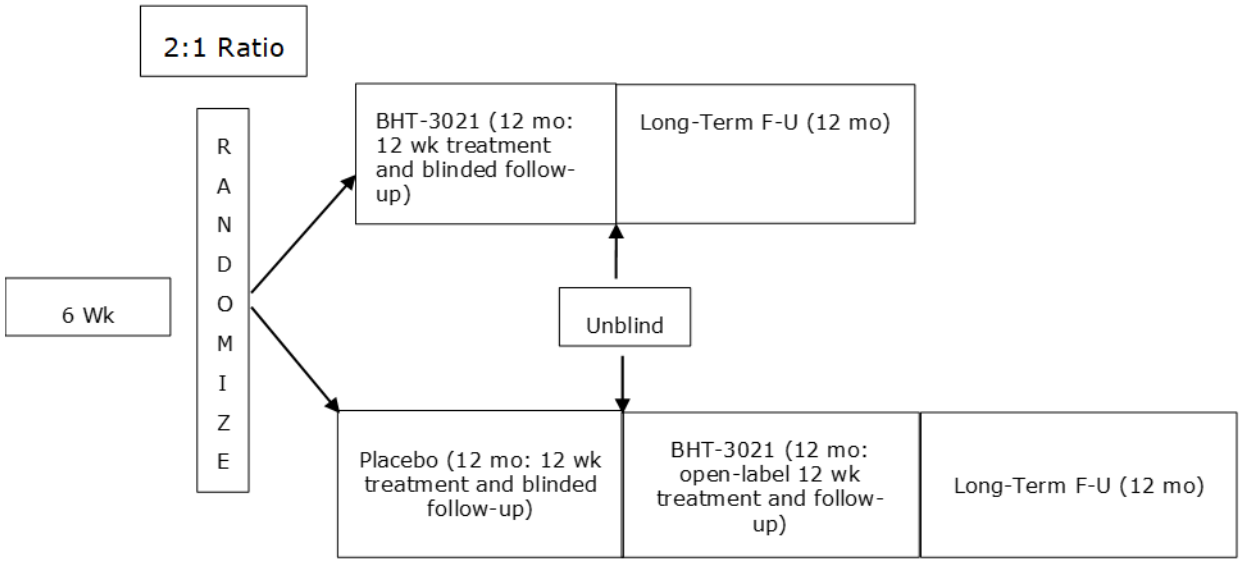
1.1 Background

The study discussed below was conducted by Bayhill Therapeutics with the investigational product designated as BHT-3021 (Roep et al. 2013). BHT-3021 was acquired from Bayhill by Tolerion, Inc. and has been renamed TOL-3021. Bayhill Therapeutics conducted a clinical randomized dose escalation study in which 80 subjects with established T1D diagnosed within 5 years were enrolled in a randomized dose escalation study to evaluate treatment with 0.3 mg, 1.0 mg, 3.0 mg, 6.0 mg BHT-3021 or placebo administered with a weekly intramuscular (IM) injection for 12 weeks.

Subjects were screened for eligibility within 6 weeks prior to randomization. Subjects were randomized to BHT-3021 or placebo in a 2:1 ratio and entered the Blinded Treatment Period, when BHT-3021 or placebo was administered via weekly IM injection for 12 weeks (Weeks 0 to 11). Four weeks after the last dose of BHT-3021 or placebo (Week 15), subjects underwent a complete evaluation for safety, pancreatic function, and anti-insulin responses. Subjects were monitored for safety and immune response in a blinded fashion for 12 months after the first dose of BHT-3021 or placebo (the Blinded Evaluation Period). Each subject's treatment assignment was then unblinded. Subjects who received BHT-3021 were invited to enter a 12-month Long-Term Follow-Up (LTFU) Period, during which they were monitored for delayed adverse events, pancreatic function, and immune response. Subjects who received placebo were eligible for cross over to receive 12 weeks of treatment with BHT-3021 in an open-label manner. The dose of BHT-3021 during the Open Label Cross Over Period was the "best dose," defined as the dose or doses already administered in the clinical trial that the Data Safety Monitoring Board (DSMB) found to have an acceptable safety profile and which the Sponsor determined at the time of cross over to present the best balance of safety, biological activity (immune response), and/or efficacy. More than one dose could have been designated as a "best dose," as long as all doses presented comparable safety and efficacy profiles. Cross over subjects were fully evaluated at the end of the dosing period (Week 15), after which they entered the Open Label Evaluation Period that lasted for 12 months after the first dose of BHT-3021. Finally, the subjects were entered in the 12-month LTFU Period.

Out of the eighty subjects enrolled in the study, the initial 9 subjects were enrolled into an open-label cohort. After completion of the dose-finding phase of the study (Dose Escalation Phase), additional subjects were enrolled to expand one or more dose cohorts in order to obtain additional safety and efficacy data (Expansion Phase). A schematic of the study design is presented in Figure 1 below.

Figure 1. Study Schematic Protocol BHT-3021



The dose escalation portion of the study enrolled subjects sequentially into the 1.0 mg cohort, followed by the 3.0 mg cohort, and then the 0.3 mg and 6.0 mg dose level cohorts concurrently. All cohorts were randomized in an active: placebo 2:1 ratio. After the dose escalation enrollment was complete, additional subjects were randomized (active: placebo 2:1) into the Expansion Cohort to receive BHT-3021 at doses of 0.3 mg, 1.0 mg, or 3.0 mg, or to placebo. Table 1 shows the number of subjects randomized by study phase and dose.

Table 1. Subject Randomization by Study Phase and Dose

	Number of Subjects Randomized			
Dose Administered	**Dose Escalation Phase**	**Expansion Phase**	**Total in Cohort**	**Placebo Subjects Crossed Over**
0.3 mg	6	8	14	
1.0 mg	10	8	18	7
3.0 mg	8	6	14	6
6.0 mg	8	0	8	
Placebo	16	10	26	
Total	48	32	80	13

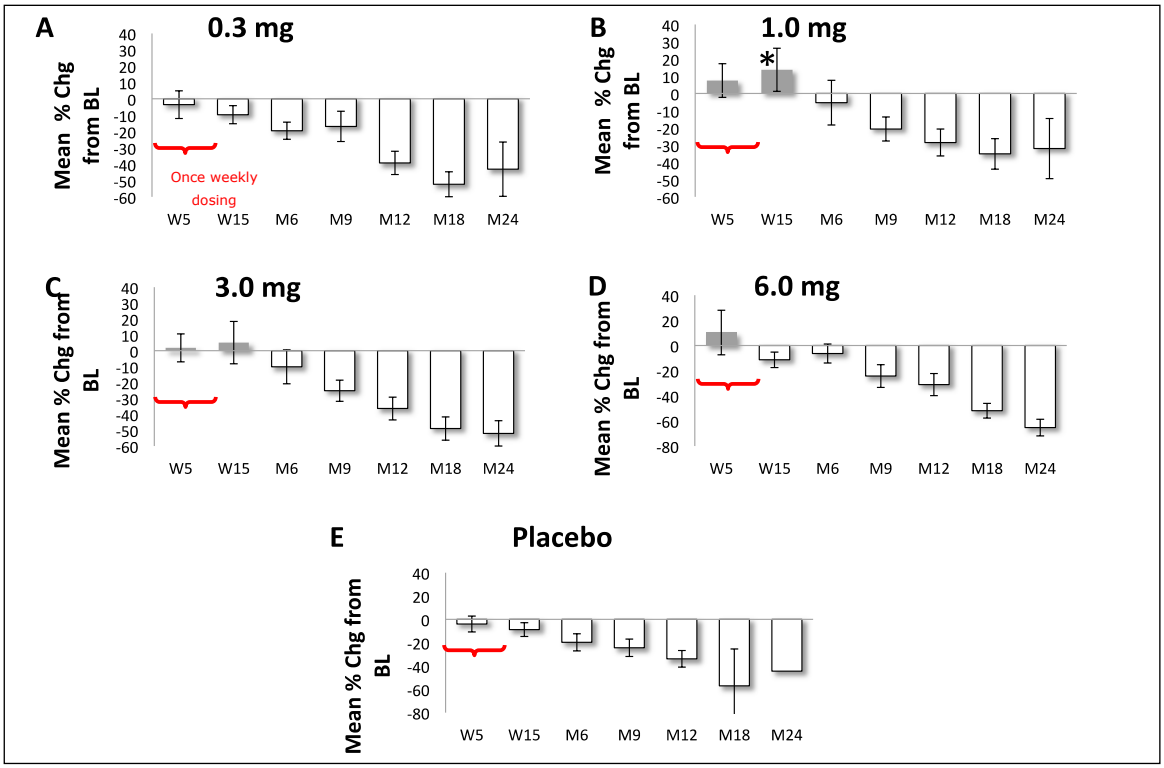
Subjects were required at screening to have a stimulated C-peptide level of ≥0.20 nmol/L and the presence of antibodies to at least one of the following: insulin andGAD65, or IA-2. They also had to agree to intensive management of diabetes with a goal of HbA1c ≤ 7.0%. While the primary objective of the study was to evaluate the safety of BHT-3021, exploratory efficacy outcomes of stimulated C-peptide, insulin requirements, glucose levels, and HbA1c were included. Additionally, immunological outcomes of insulin, GAD65, and IA-2 antibodies, as well as T-cell response to diabetes-related antigens, were measured. After completion of the 12-week blinded treatment and overall blinded evaluation at Week 15, subjects entered a long-term follow-up period of 12 months from initial dosing, with visits at 6, 9, and 12 months, during which investigators and subjects remained blinded. Subjects randomized to placebo had the option to receive open-label active treatment with BHT-3021 1.0 mg or 3.0 mg and proceeded with 12 weeks of treatment and follow-up at Week 15 as in the original study design. Subjects were then followed for the 12-month post-treatment evaluation.

BHT-3021 was well tolerated at all doses studied. There was no suggestion of drug-related toxicity at any dose of BHT-3021 studied based on the severity and relationship of reported adverse events (AEs) graded according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE). A high percentage of subjects experienced at least 1 treatment emergent adverse event (TEAE) during the study, but a relatively small percentage of these were Grade 3 or higher. Nearly all TEAEs reported were of mild severity and half or more were considered unrelated to treatment. Thirteen TEAEs of Grade 3 or higher were all considered unrelated to treatment and none of the events required expedited reporting.

The ANA and anti-dsDNA results suggest that BHT-3021 administration is not associated with an immune response against DNA.

C-peptide, which is an important surrogate marker for pancreatic secretion of insulin, was measured at Week 5 during treatment and at Week 15 (4 weeks following treatment). Measurements were repeated at 6 and 12 months during the LTFU. As shown in Figure 2, C-peptide levels increased over baseline levels, most noticeably in the BHT-3021 1.0 and 3.0 mg treatments groups compared to placebo. At Week 15, the increase over baseline seen at 1.0 mg was significant compared to placebo ($p<0.026$), with an increase of 19.5% compared to a decrease 8.8% in placebo. In subjects who responded with an increase over baseline in C-peptide at Week 15, 8 of 15 subjects treated with 1.0 mg BHT-3021 continued to show an increase of C-peptide at 6 months, while 9 of 17 subjects treated with 3.0 mg showed an increase at 6 months.

Figure 2. Mean Percent Change in C-Peptide AUC



HbA1c levels varied at baseline, reflecting differences among subjects in glycemic control, but remained fairly constant through Week 15. Overall, subjects treated with BHT-3021 tended to have lower HbA1c levels at Week 11 compared to placebo ($p<0.08$). Levels tended to rise after that with no significant differences among treatment groups. Use of insulin fell during dosing in the 1.0 mg treated group but was not statistically significant compared to placebo. Insulin use was stable among the other groups during dosing. Usage in all groups increased during follow-up, likely reflective of the increases in HbA1c and/or declining endogenous insulin secretion.

Immunological studies were conducted to quantify changes in islet-specific $CD8^+$ T cells during treatment. Data from 41 of 80 subjects were available at baseline and at least 1 other data point after treatment for this analysis. Results of these investigations indicated that BHT-3021 induced antigen-specific reductions in $CD8^+$ cells reactive to proinsulin but not to other antigens, including viral antigens. The magnitude of the reduction was inversely correlated with the improvement in C-peptide.

Autoantibodies to pancreatic antigens were measured at baseline and Week 15. Few changes in antibody status between baseline and Week 15 were noted. One placebo-treated subject converted from negative to positive for GAD65. Two active- and placebo-treated

subjects converted from negative to positive for insulin antibodies. An evaluation of C-peptide in these subjects showed no consistent changes that correlated with induction of IAs. No subjects converted from negative to positive for IA-2.

Based on analysis of serum C-peptide levels, laboratory data, and antibody responses to pancreatic autoantigens, there is no evidence that any of the doses of BHT-3021 caused an increase in autoimmunity with resultant acceleration of pancreatic beta cell loss in any subject. An increase in autoimmunity is a theoretical concern with a DNA vaccine; it is reassuring that this was not seen in this study of 80 subjects dosed for up to 12 weeks with BHT-3021. These results provide preliminary evidence that TOL-3021 may be efficacious in the preservation of ß-cell function in T1D subjects.

The study also suggested a good safety profile, which was just as important as the efficacy findings. No deaths were reported in the study. Serious Adverse Events (SAEs) that were reported were all considered unrelated to treatment and included the following: 2 subjects treated with 3.0 mg (diabetic ketoacidosis, Grade 1; infection/abscess natal cleft, Grade 3), 1 treated with 1.0 mg (hemiparesis, Grade1) and 4 treated with placebo (abdominal pain, Grade 3; elevated blood pressure and premature uterine contraction, Grade 1; migraine, Grade 1; neurologic presentation secondary to a primary cardiac abnormality, Grade 1). Two subjects treated with 3.0 mg discontinued the study due to possibly related AEs of headache and vaginal candidiasis. An overall summary of TEAEs can be found in Table 2 and a summary by MedDRA Body System in Table 3.

Table 2. TEAE Overall Summary by Subject

TEAEs by Subject	**0.3 mg n=14 n (%)**	**1.0 mg n=18 n (%)**	**3.0 mg n=14 n (%)**	**6.0 mg n=8 n (%)**	**Placebo n=26 n (%)**
Any TEAE	12 (85.7)	18 (100.0)	11 (78.6)	7 (87.5)	25 (96.2)
≥ Grade 3	0 (0.0)	4 (22.2)	2 (14.3)	3 (37.5)	4 (15.4)
Possibly Related	5 (35.7)	6 (33.3)	6 (42.9)	4 (50.0)	6 (23.1)

Table 3. TEAE Summary by MedDRA Body System

MedDRA Body System	**0.3 mg n=14 n (%)**	**1.0 mg n=18 n (%)**	**3.0 mg n=14 n (%)**	**6.0 mg n=8 n (%)**	**Placebo n=26 n (%)**
Subjects with Any TEAE	12 (85.7)	18 (100.0)	11 (78.6)	7 (87.5)	25 (96.2)

MedDRA Body System	0.3 mg n=14 n (%)	1.0 mg n=18 n (%)	3.0 mg n=14 n (%)	6.0 mg n=8 n (%)	Placebo n=26 n (%)
Blood and Lymphatic System Disorders	1 (7.1)	0 (0.0)	3 (21.4)	0 (0.0)	1 (3.8)
Cardiac Disorders	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (3.8)
Ear and Labyrinth Disorders	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)
Eye Disorders	1 (7.1)	2 (11.1)	1 (7.1)	0 (0.0)	2 (7.7)
Gastrointestinal Disorders	2 (14.3)	6 (33.3)	5 (35.7)	1 (12.5)	6 (23.1)
General Disorders and Administration Site Conditions	2 (14.3)	1 (5.6)	1 (7.1)	3 (37.5)	1 (3.8)
Immune System Disorders	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (15.4)
Infections and Infestations	10 (71.4)	13 (72.2)	9 (64.3)	6 (75.0)	13 (50.0)
Injury, Poisoning, and Procedural Complications	3 (21.4)	3 (16.7)	3 (21.4)	2 (25.0)	4 (15.4)
Investigations	1 (7.1)	1 (5.6)	2 (14.3)	1 (12.5)	3 (11.5)
Metabolism and Nutrition Disorders	3 (21.4)	2 (11.1)	3 (21.4)	3 (37.5)	12 (46.2)
Musculoskeletal and Connective Tissue Disorders	2 (14.3)	1 (5.6)	4 (28.6)	1 (12.5)	3 (11.5)
Nervous System Disorders	4 (28.6)	6 (33.3)	5 (35.7)	2 (25.0)	8 (30.8)
Psychiatric Disorders	0 (0.0)	0 (0.0)	2 (14.3)	0 (0.0)	1 (3.8)
Renal and Urinary Disorders	1 (7.1)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)
Respiratory, Thoracic, and Mediastinal Disorders	1 (7.1)	3 (16.7)	4 (28.6)	1 (12.5)	8 (30.8)
Skin and Subcutaneous Tissue Disorders	3 (21.4)	4 (22.2)	3 (21.4)	2 (25.0)	8 (30.8)
Surgical and Medical Procedures	0 (0.0)	1 (5.6)	0 (0.0)	0 (0.0)	1 (3.8)

1.2 Rationale for the Study

The phase 2a study (BHT-3021) as discussed in the Background (Section 1.1) provided evidence of the safety and efficacy of TOL-3021. None of the SAEs reported were

determined to be related to the study drug at any of the doses tested. Most of the TEAEs reported were mild. Evaluation of the injection site reactions revealed only one report of an injection site reaction and reports in four subjects of injection site pain, providing evidence that TOL-3021 administered as an IM injection is well tolerated.

Given the evidence from the phase 2a study supporting both safety and efficacy, the SUNRISE study is being conducted to further evaluate the safety and continue to explore the efficacy of TOL-3021 1.0 mg in subjects with new onset or established T1D. The SUNRISE study will also enable enrollment of adolescents (aged 12.0 to <18.0 years) in SUNRISE and other TOL-3021 studies.

The treatment effect of TOL-3021 dosing over 24 weeks and subsequently over 52 weeks on preserving endogenous insulin secretion as reflected by C-peptide secretion and its effect on other measures of efficacy will be explored. The mean C-peptide area under the curve (AUC) during a 4-hour mixed-meal tolerance test (MMTT) will be evaluated during the study at Weeks 12, 16, 24, and 52. This is the appropriate outcome for both clinical and regulatory purposes (Palmer et al. 2004). C-peptide is a useful and widely used method of assessing pancreatic ß-cell function (Jones and Hattersley 2013). C-peptide is the part of proinsulin that is cleaved prior to co-secretion with insulin in equimolar amounts. Unlike secreted insulin, which is variably cleared by the liver, C-peptide passes through the liver without any uptake. C-peptide is cleared in the peripheral circulation at a constant rate and has a half-life of 20-30 minutes, while insulin has a half-life of only 3-5 minutes. C-peptide is also not contained in the insulin therapeutic products subjects will receive to manage their diabetes. For these reasons, C-peptide is widely used as the preferred measure of endogenous insulin secretion (Yosten et al. 2014, Leighton et al. 2017).

The most reliable measurement of endogenous insulin secretion and ß-cell function is measurement of C-peptide under standardized conditions. The amount of preserved C-peptide is positively correlated with improved clinical outcomes. C-peptide levels post-stimulation are a validated means of assessing endogenous insulin secretion. Sensitive, reproducible assays for measuring C-peptide are readily available (Jones and Hattersley 2013).

In 2001, the National Institutes of Health (NIH) established the Type 1 Diabetes TrialNet to conduct studies of therapies aimed to preserve ß-cell function in subjects with recently diagnosed T1D and to prevent diabetes in subjects at increased risk of future T1D. TrialNet has adopted C-peptide as the principal outcome measure for clinical trials of interventions against T1D autoimmunity (Palmer et al. 2004). In the SUNRISE study, the change in C-peptide AUC during the MMTT at baseline, during treatment, and at the end of the study end will be compared between the control group and those receiving active therapy in patients who have been diagnosed with T1D within 5 years of study enrollment. The trials

may also demonstrate a clinically meaningful reduction in mean daily insulin requirements accompanied by similar or better glycemic control in those actively treated compared to the control arm (FDA 2008). FDA's position on the primary outcome would presumably apply also to trials of established T1D patients.

2 OBJECTIVES

2.1 Primary Efficacy Objective.

To evaluate the effect of TOL-3021 dosing over 24 weeks on preserving endogenous insulin secretion as reflected by C-peptide secretion, and its effect on other measures of efficacy in patients with Type 1 Diabetes Mellitus (T1D).

2.2 Safety Objective

To evaluate the safety of TOL-3021 administered as weekly intramuscular (IM) injections over 52 weeks in patients with Type 1 Diabetes Mellitus (T1D).

3 TRIAL DESIGN

The SUNRISE study is a prospective, multi-center, randomized, placebo-controlled trial in subjects aged 12.0 to <41.0 years diagnosed with T1D, as defined by ADA criteria, and within 5 years of diagnosis. Time of diagnosis is defined as the first day of insulin administration. The study is triple-blind through the analysis at Week 24, after which the Sponsor will be group unblinded. The study will continue through Week 52 as double-blind with site staff and subjects blinded. Subjects will be stratified by duration (zero up to 1 year and 1 year up to five years) to ensure balance of disease duration across treatment and placebo groups in each strata. For analytical purposes, all subjects12-<41 will be considered cohort A, subjects aged 12-<18 will considered cohort B and subjects aged 18-<41 will be considered cohort C. For subjects aged 12-<18 (Cohort B), dosing will be staggered with an initial 6 subjects aged 14-<18 being enrolled with the last subject a having a minimum of 2 injections with at least 1 week follow-up after the 2nd injection. Safety data from this cohort will be evaluated before opening the study subjects 12 and older. Subjects should be randomized no sooner than 6 weeks after diagnosis, unless blood glucose is adequately controlled as indicated by time in range >55% with CGM. Screening assessments will include a physical examination, a fundus photography examination, chemistry and hematology safety labs, urinalysis, urinary protein screen (if positive a 24-hour collection for urine protein and creatinine will obtained), HbA1c, presence of T1D antibodies, and a MMTT. Up to 99 qualified subjects who meet all selection criteria will be randomized in a 2:1 ratio to treatment with TOL-3021 or placebo and treated for 52 weeks (Figure 3). Subjects will agree to diabetes management during the

study with the goal of maintaining HbA1c levels of approximately 7.0% without frequent episodes of hypoglycemia.

Study drug treatments will be administered via an IM injection into a large muscle every week for 52 weeks. Initial treatments will be administered at the clinical site at randomization and at the Week 1 and Week 2 visits. During those visits, subjects and/or a caregiver will be trained to administer study drug at home in subsequent weeks. Study drug will be dispensed at the Week 2 visit for Week 3 administration at home (with the exception of the first 6 subjects aged 14-<18 to be enrolled in cohort B who will have drug administered at the clinical site), and at Weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 for at home administration.

Subjects will have screening and randomization visits, followed by regular visits to the clinic, for efficacy evaluations and safety assessments throughout the 52-week study. A 4-hour MMTT will be conducted during screening, as part of a single-day visit or conducted on a separate day. If the screening visit procedures will be conducted in a single day the subject must be using their own CGM, and able to share that data with site coordinator to confirm if time in range has been met for 3 of 5 consecutive or non-consecutive days prior to confirmation of a single screening visit. Subjects will be provided with instructions for preparing for the MMTT prior to the visit when it will be conducted. Subjects with peak C-peptide during the screening 4-hour MMTT < 0.150 nmol/L will not be enrolled. The randomization visit must be at least 3 days after the screening MMTT. The screening results will be the baseline value for the MMTT. Continuous glucose monitoring (CGM) will be initiated within 5 days prior to the screening MMTT visit and continued through Week 52. If CGM recordings are inadequate during week 50-52, CGM may be continued until the Week 54 visit. The study will allow subjects to be unblinded to interstitial glucose data from the CGM device to minimize any risk to subjects. Site research staff will train subjects or their caregivers on appropriate use and associated risks of using a CGM device.

A Data Safety Monitoring Board (DSMB) was established prior to study initiation to review data throughout the study, including the 12-week data described below.

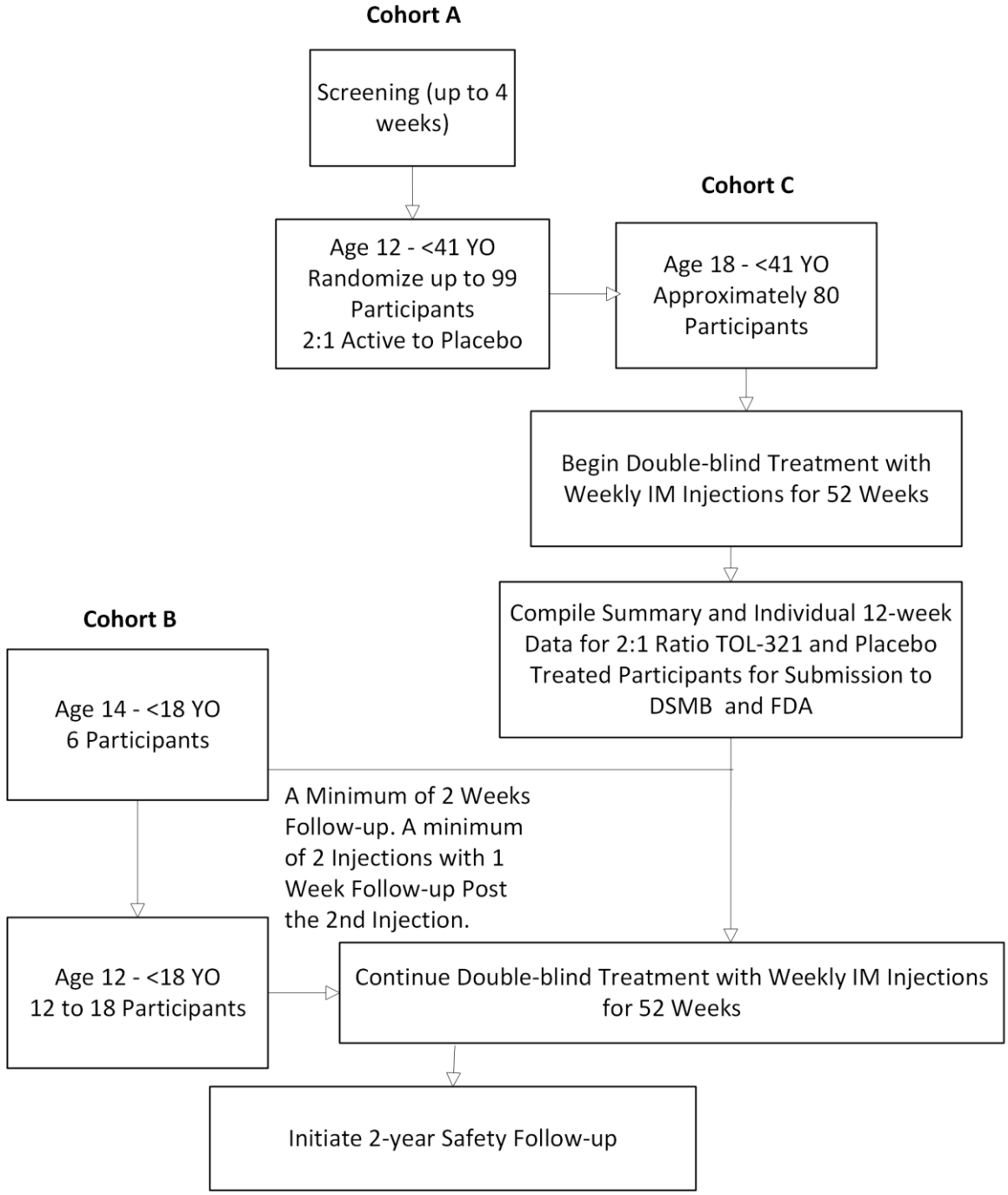
When the first 30 treated subjects completed the Week 12 visit, all available data was compiled and provided to the DSMB to consider if the enrollment of adolescent subjects in TOL-3021 studies can be recommended. Summary and individual subject data were provided for DSMB and FDA review. The DSMB~~,~~ confirmed that the protocol can be amended to include subjects aged 12 - < 18 years of age and submitted to FDA. If no objection is received from regulators and IRBs, sites will be notified that they may begin enrollment of subjects aged 12 - <18.

Subjects and investigators will be blinded to treatment assignment throughout the study. Selected Emmes staff will have access to treatment assignments. The primary data analysis will be conducted on data collected through Week 24. Cohorts B and C may be analyzed

separately. An interim analysis will be completed after the first 30 patients have completed 24 weeks. Results of the interim analysis will be shared with the DSMB and with a limited number of Tolerion staff, not directly involved in running the trial. Unblinded data summaries including safety outcomes and efficacy assessments will be prepared for the sponsor during the study using all data collected through Week 36. Final analysis of the data will be conducted at 52 weeks. The data summaries are unblinded to Sponsor and Emmes, the CRO, only.

Subjects will continue in the study for a 2-year safety follow-up period after completion of the 52-week double-blind study. During this 2-year follow-up subjects will have clinic visits at 18, 24, and 36 months from their first dose in the double-blind study to evaluate safety. At each of these follow-up visits a MMTT will be administered. Subjects may also be offered the opportunity to continue on study medication in a follow-on protocol for one or more years. Any follow-on study will continue to monitor subject safety in line with the requirements of the SUNRISE protocol.

Figure 3. Study Schematic



4 OUTCOMES

4.1 Safety Outcomes

The following will be tracked and monitored through the course of the study

- Clinical laboratory tests (hematology, chemistry, urinalysis);
- Urine pregnancy test (UPT) for women of childbearing potential (WOCBP);
- Concomitant medications;
- Collection of adverse events (AEs);
- Vital signs;
- Injection site reactions;
- Severe hypoglycemia or hyperglycemia events monitored by CGM;
- Events of special interest:
    - Systemic or hypersensitivity reactions associated with injection, which consist of fever, chills, headache, nausea, vomiting, and/or other signs and symptoms, such as anaphylaxis, wheezing dyspnea, urticaria, and hypotension

4.2 Efficacy Outcomes

4.3 Primary Efficacy Outcomes

The primary outcome is the TOL-3021 treatment effect by a repeated measures analysis of change from baseline in the log-transformed MMTT C-peptide AUC at 12, 16, and 24 weeks of treatment.

4.4 Secondary Efficacy Outcomes

Secondary outcomes will include the treatment effect on:

Rate of clinically important hypoglycemia, defined as measured glucose value of <54mg/dL (3.0 mM/L) over each approximately 12-week period ending at Weeks 12, 24, 36, and 52:

- A single blood glucose level.
- By CGM, ≥10 consecutive minutes with glucose <54mg/dL.

Total daily insulin requirements in units per kilogram (kg) body weight

- HbA1c.

- Time in glycemic range 70 - 180 mg/dL

4.5 Other Secondary Outcomes

The TOL-3021 treatment effect—

On a repeated measures analysis of change from baseline in the log-transformed MMTT C-peptide area under the curve (AUC) at Weeks 12, 16, 24, 52.

On number of times the CGM reports glucose levels of <70 and <55 mg/dL.

On a clinical responder analysis that will be undertaken to further characterize the treatment effect on a clinical level. A positive responder outcome will be defined as no change or an increase in C-peptide AUC from baseline between treatment and placebo at Weeks 12, 16 and 24. Upon completion of 52 week data, a similar analysis will include the 52-week data.

On non-fasting or fasting C-peptide (single test from baseline at Weeks 12, 16, 24, and 52.

On proportion of subjects in each treatment arm with HbA1c levels less than 6.5% at Week 52.

On CGM parameters:

- Time >180 mg/dL
- Time >250 mg/dL
- Mean Glucose Coefficient of Variation
- Low Blood Glucose Index (LBGI)
- Glucose below 70 mg/dL
- Area Over the Curve (AOC_{70})

On other measures of hypoglycemia:

- Severe hypoglycemia (SH) events (impaired or loss of consciousness requiring assistance of another).
- Documented symptomatic hypoglycemia (an event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration <70 mg/dl (3.9 mmol/L).
- Total time <70 mg/dL by CGM.
- Nocturnal hypoglycemia, i.e., severe or documented symptomatic episodes (as defined above) occurring after the subject has retired for the primary sleeping period.

Immunologic:

Quantum dot responses within the qualifying subpopulation to confirm induction of specific autoantigen tolerance

Comparison of quantum dot responses within the qualifying subpopulation with clinical outcomes to confirm correlation with specific autoantigen tolerance

Determine effect of treatment on and predictive value of:

- Regulatory/protective humoral immune response to proinsulin/insulin
- Serum insulin autoantibody affinity for subjects
- Insulin autoantibody isotypes (IgA and IgM) and IgG subclasses
- Serum insulin, glutamic acid decarboxylase, IA-2, and ZnT8 antibodies by radio-binding assay (RBA) assay
- Competition assays of serum insulin and proinsulin IgM and IgG antibodies

5 STUDY POPULATION

5.1 Number of Subjects

Up to 99 subjects will be randomized in a 2:1 ratio of treatment with TOL-3021 to placebo with a minimum of 18 and a maximum of 24 subjects aged 12 and <18 years of age.

5.2 Selection Criteria

5.2.1 Inclusion Criteria

1. Diagnosis of Type 1 Diabetes Mellitus based on ADA criteria and within 5.0 years from diagnosis, defined as the first day of insulin administration.
2. Age at randomization of 12.0 – <41.0 years of age[1].
3. Adequate glycemic control as defined by HbA1c ≤7.9% based on point-of-care or local lab measurement and time in glycemic range (70-180 mg/dL) >55% by CGM recording over 3 or more consecutive or non-consecutive days within 5 days prior to baseline mixed meal tolerance test (MMTT).
4. On insulin therapy (total insulin dose >0.125 U/kg BW)
5. Presence of antibodies to at least one of the following antigens: GAD65, IA-2, ZnT8, or insulin if obtained within 10 days of the onset of exogenous insulin therapy, or documentation of positive antibodies. In the absence of a positive result for one of the specified antibodies, a diagnosis of T1D per ADA guidelines.

6. Peak C-peptide during screening 4-hour mixed meal tolerance test (MMTT) ≥ 0.150 nmol/L.
7. Willingness to wear the Dexcom G6 continuous glucose monitoring (CGM) device and use according to instructions including recording of total daily insulin dose taken most of each day from screening to end of treatment period.
8. Written informed consent and, for subjects aged 12-<18 years of age, patient assent and parental or guardian consent, including authorization to release health information.
9. Willingness and ability of subject to comply with all study procedures of the study protocol, including attending all clinic visits.

5.2.2 Exclusion Criteria

1. Receiving a dose of acetaminophen >4,000 mg per day.
2. Body Mass Index (BMI) >32 kg/m^2 for patients 18 and older (>85th percentile for ages 12-17)
3. Previous immunotherapy for T1D within 2 years of enrollment.
4. Diagnosis of liver disease or hepatic enzymes, as defined by ALT and/or AST ≥ 2.5 times the upper limit of normal (ULN).
5. Hematology: white blood cells (WBC) <3 x 10^9/L; platelets <100 x 10^9/L; hemoglobin <10.0 g/dL. (Low WBC values may be repeated every 3-7 days, and results to be discussed with the Medical Monitor)Any underlying conditions likely to impact red blood cell turnover.
6. Latent autoimmune diabetes of adults (LADA), which is generally associated with preceding history and treatment of T2D with medications typically used for treatment of T2D for more than 30 days.
7. Monogenic diabetes (MODY)
8. Estimated glomerular filtration rate (eGFR) <60 ml/min, for ages 18-<41, and <75 ml/min per 1.73 m2 for ages 12-<18.
9. History of malignancy, except for cancers in remission >5 years, or basal cell or in situ squamous cell carcinoma of the skin.
10. Significant cardiovascular disease (including inadequately controlled hypertension), history of myocardial infarction, unstable angina, use of anti-anginal medicines (e.g., nitroglycerin), or abnormal stress test, which, in the opinion of the Principal Investigator (PI), would interfere with participation in the trial.

11. Immunosuppressive therapy (systemic corticosteroids, cyclosporine, azathioprine, or biologics) within 30 days of screening.
12. Current or prior (within the last 30 days) use of metformin, sulfonylureas, glinides, thiazolidinediones, GLP1-RAs, DPP-IV inhibitors, pramlintide, or SGLT-2 inhibitors.
13. Current use of verapamil or α-methyldopa.
14. History of any organ transplant, including islet cell transplant.
15. Asthma that requires oral glucocorticoid therapy. Inhaled glucocorticoid therapy is permitted.
16. Active autoimmune or immune deficiency disorder including rheumatoid arthritis, moderate-to-severe psoriasis, inflammatory bowel disease, and other autoimmune conditions that may require treatment with TNF or other biologics. Permitted autoimmune disorders include T1D or well-controlled autoimmune conditions (e.g., thyroid disease, celiac disease, and sarcoidosis, all with stable non-immunosuppressive medications for the past 30 days).
17. Thyroid-stimulating hormone (TSH) at screening >7.5 mIU/L,for ages 18-<41 years old and > 3.6mIU/L for ages 12-<18 years old.
18. Adrenal insufficiency not adequately controlled on stable replacement glucocorticoid therapy.
19. Moderate non-proliferative retinopathy (NPDR) or proliferative retinopathy
20. Evidence of infection with HBV (as defined by hepatitis B surface antigen, HBsAg), HCV (anti-HCV antibodies), or HIV.
21. Subject is breastfeeding.
22. Positive urine pregnancy test at screening or at any time during the study (pregnancy tests must be performed as per the visit schedule): Females of childbearing potential must be excluded if they have a positive urine pregnancy test at screening or randomization or if they are not using medically acceptable methods of birth control. Acceptable methods of birth control include oral or transdermal contraceptives, condom, spermicidal foam, IUD, progestin implant or injection, abstinence, vaginal ring, or sterilization of partner. The reason for non-childbearing potential, such as bilateral tubal ligation, bilateral oophorectomy, hysterectomy, or 1 year or more postmenopausal must be specified in the subject's Case Report Form (CRF).
23. Males of reproductive potential who are unwilling to use medically acceptable birth control, unless the female partner is postmenopausal or surgically sterile.

24. Any social condition or medical condition that would, in the opinion of the PI, prevent complete participation in the study or would pose a significant hazard to the subject's participation.
25. Anticipated major surgery during the duration of the trial, which could interfere with participation in the trial.
26. History of drug or alcohol dependence within 12 months of screening.
27. Psychiatric disorder that would prevent subjects from giving informed consent.
28. Household members of current participants in this protocol.
29. Subjects who are not fluent in the English language.
30. Participation in other studies involving the administration of an investigational drug or experimental device, including the administration of an experimental agent for T1D within 30 days of screening, or use of an experimental therapeutic device for T1D within 30 days prior to screening. Subjects previously treated with diagnostic devices are not excluded.
31. Any current use of biotin or biotin containing supplements.

5.3 Discontinuation Criteria

5.3.1 Withdrawal and Early Discontinuation from the Study

At any point after randomization, a subject may withdraw consent to be administered study medication or followed. A subject withdrawing consent to be administered study medication would be encouraged to otherwise remain in the study and to complete as many procedures as the subject is willing. The subject may withdraw entirely from study participation at any time. If the subject withdraws consent and discontinues from the study, the PI will attempt to determine the reason for discontinuation and record the reason in the subject's source documents and in the eCRF. In the event of early discontinuation from study drug, the subject will be asked to return to the site to complete the assessments specified in the End-of-Study Visit (Weeks 52 and 54).

The PI can discontinue study drug in any subject at any time if medically necessary. The PI, in consultation with the Medical Monitor, or the Medical Monitor may exercise his or her medical judgment to discontinue a subject's treatment in the study due to clinically significant changes in any clinical or laboratory parameter. Prior to discontinuing a subject from study participation, the PI will discuss his or her intentions with the Medical Monitor.

The discontinuation eCRFs (End of Medication and Study Termination) must be completed for every subject who received study medication regardless of the subject's study

completion status. The primary reason for any early discontinuation should be indicated on these eCRFs. Standard categories of early termination include:

- *Adverse event*: Reasons for discontinuation may include serious and non-serious AEs regardless of relation to study medication, or decision on the part of the subject to discontinue due to an AE.
- *Investigator judgement*: Clinical observations or laboratory results led the PI, in his or her medical judgement, to conclude that discontinuation was in the best interest of the subject. The reason should be specified in the eCRF.
- *Death:* The subject died.
- *Withdrawal of consent*: The subject decided to withdraw from further participation in the study in the absence of a medical need as determined by the PI. The reason for discontinuation should be recorded on the eCRF.
- *Protocol deviation*: The subject 's findings or conduct failed to meet the protocol entry criteria post-randomization or subject failed to adhere to the protocol requirements. The deviation necessitated premature termination from the study.
- *Lost to follow-up*: The subject failed to attend visits and study personnel were unable to contact the subject after two attempted phone calls and a registered letter.
- *Other*: The subject was terminated for a reason other than those listed above. The reason should be specified in eCRF.

The Sponsor reserves the right to terminate the study at any time, especially if the scientific question is no longer relevant or if the study objectives will not be met. All data normally collected at completion of the study must be collected either at the time of the subject's early termination or on or before the scheduled study closeout visit. Reasons for termination of the study include:

- AEs occur with such severity and frequency that adherence to the proposed schedule cannot be continued;
- Risks that cannot be adequately quantified;
- Failure to remedy deficiencies identified through site monitoring, failure to meet identified Sponsor performance standards, or substandard data;
- Sponsor decision to discontinue the development of the product or the indication under study.

5.3.2 Trial Stopping Rules

Study enrollment or study drug administration may be suspended if certain events occur during the trial. The chairman of the DSMB will be notified that a review of safety data

may be required to determine procedures that need to be implemented if any of the following occur:

- Any death deemed to be related to study therapy
- More than one hospital admission of the same event based on MedDRA preferred term for an unexpected treatment-related SAE
- Three of the first 10 subjects, or 30% thereafter, require discontinuation of study medication for the same or similar serious adverse event based on MedDRA preferred term or at the discretion of Sponsor and/or Medical Monitor

5.4 Data Safety Monitoring Board (DSMB)

Well qualified members were selected to serve on the Data Safety Monitoring Board (DSMB). The DSMB was established prior to study initiation to review data throughout the study, including review of 12-week data to enable the enrollment of adolescent subjects in TOL-3021 studies. The DSMB recommended that the protocol be amended to include subjects aged 12 - <18 years of age and this was submitted to FDA. If no objection is received from FDA, the protocol will include this subgroup and sites will be notified that they may begin enrollment of subjects aged 12 - <18. Once amended, data from adolescent participants will be summarized and presented separately from data on adult participants in all subsequent DSMB review meetings. The DSMB will also review data from an interim analysis when the first 30 subjects have completed 24 weeks of treatment.

The Sponsor and Emmes will support the DSMB in completing its charter and in all aspects of fulfilling its responsibilities. The DSMB will monitor the progress of the study, review safety data, and make recommendations regarding continuation, termination, or modification of the study. Specific items reviewed by the DSMB will include, but will not be limited to: study demographic information; interim/cumulative data for evidence of study-related AEs; protocol deviations; data quality, completeness, and timeliness of visits; factors that might affect the study outcome or compromise the confidentiality of the trial data (such as treatment and endpoint unblinding); and factors external to the study, such as scientific or therapeutic developments that may impact subject safety or the ethics of the study. The number of subjects who discontinue study treatment will also be included in the reports prepared for the DSMB. In addition to regular meetings, the DSMB chair may convene a full DSMB for review of an emergent issue.

DSMB recommendations will be carefully considered by the Sponsor. If a disagreement arises between the Sponsor and the DSMB, the Sponsor will discuss it with the DSMB in order to reach consensus. If attempts to reach a consensus fail, the Sponsor's opinion will prevail. In such situation, the Sponsor will inform the regulatory authorities, if needed.

5.5 Concomitant Medication

5.5.1 Required Medications

Subjects are required to use insulin regimens consistent with standards of care as advised by their primary physician or the PI.

5.5.2 Permitted Medications

Concomitant medications required for standard care are permitted, except for those specified in Section 5.5.3 below as prohibited medications. Use of all concomitant medications must be documented in source documents and reviewed at each visit. Medications taken within the last three months before screening will be documented in an eCRF. Subjects will be encouraged to avoid making changes to their concomitant medication regimen during their participation in the study. Any change to a subject's concomitant medication regimen after randomization will be documented in an eCRF. In addition, PIs are encouraged to avoid adding to or changing a subject's medications during study participation unless deemed medically necessary. As clinically indicated, all standard vaccinations are permitted prior, during, and after the treatment period. Preferably, vaccinations should be given in between weekly TOL-3021 dosing and in a muscle that has not been used in the previous 2 weeks for TOL-3021 administration. Hormonal replacement therapy for emergent hypothyroidism or adrenal insufficiency can be started during the course of the study if recommended by the subject's primary endocrinologist.

5.5.3 Prohibited Medications

Use of the following medications are prohibited from 30 days prior to randomization through the Week 52 final study visit:

- Systemic glucocorticoids or immunosuppressive agents (e.g., cyclosporine, azathioprine, methotrexate, infliximab, or biologic agents). A short course (<21 days) of systemic glucocorticoids for treatment of a transient condition (e.g., asthma) or replacement therapy for adrenal insufficiency is permitted.
- Medications other than insulin for glycemic control (e.g., metformin, sulfonylureas, glinides, thiazolidinediones, GLP1-RAs, DPP-IV inhibitors, pramlintide, or SGLT-2 inhibitors).
- Verapamil, α-methyldopa.
- Investigational drugs or devices.
- Acetaminophen >4,000 mg per 24-hour period.

- Biotin

5.6 Diabetes Management

Glycemic control in all study subjects will conform with an accepted standard of "intensive" management. HbA1c will be assessed every 3 months (or as per schedule of events) to evaluate metabolic control. All individuals will be encouraged to strive for targets in accordance with current ADA recommendations: HbA1c of 7% for all adult subjects and modified for younger subjects to also have an HbA1c target of 7%, preprandial glucose levels of 90–130 mg/dL, postprandial levels of less than 180 mg/dL, and bedtime levels of 110-150 mg/dL. All subjects will generally receive three or more injections of insulin daily, including short- and long-acting insulin analog products. Alternatively, subjects may use continuous subcutaneous insulin infusion by insulin pump. Consistent with ADA Standard of Care Guidelines, glucose levels should be checked multiple times daily. All subjects should properly use their CGM device every day, which includes checking the meter results frequently. On the basis of glucose measurements, the Diabetes Management Team (DMT) may advise, as may be needed, the subject's physician about possible adjustments in the insulin regimen, dietary modifications, including referral to a registered dietitian, or other approaches that the DMT believes could improve the glucose control. Subjects may be contacted by the diabetes educator or other professional at the site between visits to counsel subjects. Insulin use will be recorded daily using the CGM device and hypoglycemic events will be captured at each visit on the appropriate eCRFs. For purposes of achieving sustained good glycemic control subjects will be encouraged to record glucose, insulin dose, and dietary information, and to share this information at clinic visits. This information is for clinical care and is not to for database entry.

<u>The Diabetes Management Team</u>

The Diabetes Management Team (DMT) is a resource for ensuring that all subjects maintain good glycemic control while avoiding hypoglycemia and acidosis. The DMT could include qualified members from Emmes, Sponsor, clinical sites, or a remote group assigned by the study Sponsor to review HbA1c, blood glucose data, and adverse events (AE) associated with diabetes self-management issues, such as hypoglycemia and DKA, and be of assistance on a PRN basis when requested by PI/Study Coordinator in order to prevent poor glycemic control in subjects enrolled in this study.

6 RANDOMIZATION

Subjects will be randomized in a 2:1 ratio of treatment with TOL-3021 to placebo. Randomization will be performed via a secure electronic data capture (EDC) system,

Advantage eClinical. Randomization will be stratified by duration of diabetes: 0-<1 year and 1-5 years, to ensure balance of TOL-3021 and placebo within each strata. The randomization code will be prepared by an unblinded statistician at Emmes and will be provided to the designated distributor that will package and ship study drug to the sites. Study drug will be labeled with the blinded numeric code provided by the statistician. Advantage eClinical will assign study drug vials to each study subject.

7 UNBLINDING

The PI and site staff are blinded to subject treatment throughout the study and do not have access to the randomization code. Unblinding assignments will be maintained by the unblinded statistician at Emmes that prepared the randomization code.

In case of an unexpected and related SAE for which knowledge of the subject's treatment is required for the subject's clinical care and safety, the blind for that subject may be broken. The PI must contact the Medical Monitor with an explanation of the need to obtain the study treatment information. The Medical Monitor will confer with the Sponsor prior to the authorization of the blind to be broken. Complete documentation of breaking the blind must be recorded in the subject's source documents including the date and time the blind was broken, the reason for the decision to unblind, and the names of the personnel involved in the decision. The unblinded statistician will conduct the unblinding, notify the site PI, and also the DSMB of all such cases.

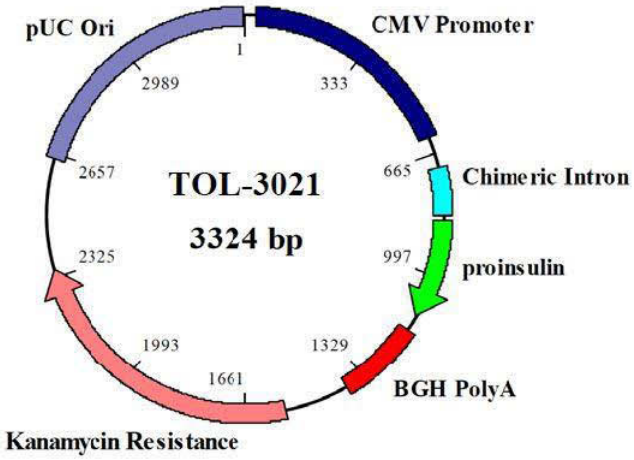
8 MATERIALS

8.1 Study Drug

TOL-3021 is a 3.3 Kb bacterial plasmid expression vector containing the coding sequences for the human proinsulin (hINS) gene. Important functional and control features of TOL-3021 include the human cytomegalovirus (CMV) immediate-early gene promoter/enhancer, the bovine growth hormone gene polyadenylation signal, the kanamycin resistance gene, and the pUC origin of replication for propagation of the vector in *E. coli*. The backbone of TOL-3021 has been modified to decrease the number of immunostimulatory CpG sequences and substitute immunosuppressive sequences (Figure 4).

Figure 4. Structural Diagram of TOL-3021

Features	
CMV Promoter	32 – 621
Chimeric Intron	702 – 835
Proinsulin	845 – 1111
BGH PolyA	1155 – 1379
Kan Resistance	1552 – 2346
pUC Origin	2646 - 3319



TOL-3021 is formulated in a phosphate-buffered, calcium-containing sterile solution for IM injection. The nominal concentration of TOL-3021 in this solution is 2 mg DNA/mL. TOL-3021 is provided in a sterile single-use 2 mL vial. Placebo is a phosphate-buffered sterile saline solution packaged in a sterile single-use 2 mL vial.

8.2 Storage, Dispensing, and Reconciliation of Study Drug

8.2.1 Receipt and Storage of Study Drug

Study drug will be supplied in individual cartons containing single-use vials. The carton and the vial are labelled with a barcode that can be scanned for maintaining inventory and dispensing records. The site will maintain shipping documents and acknowledge receipt of study drug to Emmes. Study drug will be shipped frozen and must be placed in a locked freezer in a secured location and maintained at -20 ± 5°C upon receipt until dispensing for subject treatment.

8.2.2 Dispensing of Study Drug

Study drug (active or placebo) will be labelled with required information on the vials of study drug while maintaining the study blind. Study drug will be designated by the randomization scheme for treatment of a specific subject throughout the study. Study drug will be tracked via a scannable barcode label affixed to the box containing the vial of study drug and reported in Advantage eClinical when study drug reaches the site. In addition, a tear-off label on the study drug carton will be removed and placed in the subject's source documents. An individual vial of study drug will be dispensed to the appropriate site staff

for administration to a subject at randomization, Week 1, Week 2, and Week 4 clinic visits. The study drug vial for Week 3 administration at home will be dispensed at the Week 2 visit, except for the first 6 subjects aged 14-<18 in Cohort B. These subjects will have a visit at Week 3 and will receive their study drug injection at the visit. When a subject will self-administer study drug following a clinic visit, the appropriate number of study drug vials will be dispensed for at-home use until the next clinic visit. Vials dispensed to subjects will be stored under refrigeration until use. Subjects will be given instruction on storage and administration of study drug. The PI (or designee) is responsible for maintaining inventory records, acknowledging receipt of study kits, and dispensing records that track distribution of medication to subjects. Subjects will be instructed to return used vials and any unused vials at each study visit and PIs (or designees) are responsible for recording receipt of returned used vials/unused vials for accountability.

8.2.3 Study Drug Accountability

- Drug accountability forms will be provided by Emmes to track receipt, dispensing and return of all study drug vials. Accountability forms will contain the following information:
- ID number of vials received, including date and identifying information provided on label
- ID number of vials currently stored in freezer at -20 ± 5 °C storage
- ID number of vials dispensed by date to each subject, identified by a unique subject ID number
- Non-administered disposition (e.g., wasted, broken)
- ID number of vials returned to site staff for subsequent accountability and disposal, if applicable
- ID number of vials destroyed on site

These records will be made available to the study Clinical Research Associate (CRA) for review during monitoring visits.

Emmes will provide forms in Advantage eClinical to facilitate investigational product inventory control. All investigational product accountability forms and treatment logs must be retained in the PI's permanent study file. These records must be available for inspection by the Sponsor, its designees, or regulatory agencies at any time after study completion.

The PI (or designee) must maintain 100% accountability for all investigational products received and dispensed during his or her entire participation in the study. Proper drug accountability includes, but is not limited to:

- Frequently verifying that actual inventory matches documented inventory;
- Verifying that all containers used/broken/unused/lost are documented accurately on the log;
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, the Sponsor and Emmes must be notified immediately.

At study closeout, the PI (or designee) or pharmacy will be required to reconcile all received, dispensed, and returned drug supplies.

8.2.4 Returns and Destruction of Study Drug

The site will receive instruction from the Sponsor regarding the final disposition of any remaining investigational products. The used vials must be kept separately for accountability. At completion of the study (i.e., after the Week 52 visit of the last subject), there will be a final reconciliation of investigational products shipped, investigational products consumed, and investigational products remaining. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused investigational products. All unused vials will be returned to the Sponsor or its designee. The Sponsor must approve the destruction of any study drug and receive a copy of the destruction record(s).

8.2.5 Administration of Study Drug

TOL-3021 or placebo will be administered weekly via an intramuscular injection of 0.5 mL for 52 weeks. Injections will be administered at the study site at Randomization and Weeks 1, 2, and 4 and at week 3 to the first 6 subjects aged 14-<18 years of age in cohort B. During these visits, subjects and any designated caregiver(s) will be given instruction on administration of study drug at home. Study drug injections after Week 4 (and at Week 3) will be administered at home. Subjects will be provided with all necessary supplies for administration of the injections including tuberculin syringes for drawing study drug up from vial, 23 gauge needles in the length(s) appropriate for subject age, weight, and injection site to be used (see Table 4 as a guide), alcohol wipes, gauze pads, band aids, and a sharps container. An exact number of study drug vials to cover injections over the interval to the next visit will be dispensed. Study drug will be packaged for transport at the clinic in an appropriate protective enclosure. Subjects will be instructed to place the study drug in their refrigerator in an area where it will not be disturbed. Thorough hands on training will be provided by trained study staff and will include the following:

- Locating site for injection, including the deltoid muscle in the arm and the vastus lateralis muscle in the thigh. Subjects 18 years or older may also be given the option of using the ventrogluteal site.
- Procedure for drawing study drug into syringe and attaching appropriate needle.
- Preparing the injection site.
- Administering the injection, including how to insert the needle at a 90 degree angle (straight) into the muscle with a rapid, smooth, motion all the way up to the hub of the needle, steadying the syringe, and, using gentle pressure to administer the drug over a 3 to 5 second period, withdrawing the syringe and holding a sterile gauze pad over the injection site to contain any droplets of blood.
- Disposal of the needle and syringe into a sharps container.
- Recording the date and time of the injection in the diary; subjects may also be asked to take a photograph of the injection site using a mobile phone.

Detailed written and diagrammatic instructions will be given to each subject and caregiver(s) so that they are well prepared to administer the study drug at home.

Subjects will be instructed to rotate injection sites so that the same site is not used for consecutive injections, and to avoid using the injection site arm or leg for strenuous activity during the following 24 hours. Subjects will be instructed to contact the study site immediately to report any unusual pain/tenderness, redness, swelling, bruising, burning, itching, or ulceration of subcutaneous abscess at the injection site.

Table 4. Selection of Needle Length for Study Drug Administration

Injection Site	Subject Specifics	Needle Length (inches)
Deltoid		3/4- 1
Anterolateral thigh		1
Ventrogluteal		1 – 1 ½
	≥18; >200 lbs; female	1 ½
	≥18; >260 lbs; male	1 ½

9 WARNINGS AND PRECAUTIONS

All study medication must be stored as instructed and used only by the subject participating in the study. Study drug must be administered only by IM injection according to provided instructions.

10 STUDY PROCEDURES

10.1 Study Visits

Procedures to be conducted at each study visit are listed below and shown in APPENDIX 1.

10.1.1 Screening Visit

Screening assessments and procedures may be performed in a single day or in multiple visits. Subjects should complete all screening procedures within 4 weeks of randomization even if some procedures need to be repeated. Note that subjects must be randomized within 5.0 years of T1D diagnosis, where diagnosis is defined as the first day of insulin use. All screening procedures including the MMTT could be completed in a single visit if the subject is using their own CGM and can share data with the site prior to the visit to allow site staff to ensure that the subjects meets criteria for adequate glycemic control (>55% of CGM readings within range of 70 – 180 mg/dL). In this case, subjects must be informed of the MMTT requirements in advance of the visit. Subjects must have CGM recording over 3 or more consecutive or non-consecutive days within 5 days prior to the screening MMTT to establish the adequacy of glycemic control. If preferred by the subject, or if additional time is required to complete all procedures (e.g. fundoscopic photography), additional screening visits may be scheduled as long as they fall within the screening period. If patients fail to meet the qualification criteria for HbA1c or other lab tests, the screening period may be extended an additional 4 weeks without repeating other screening tests. Randomization must occur within 4 weeks of the screening MMTT. The MMTT may be repeated to enable randomization if more than 4 weeks have elapsed since the initial screening MMTT. If a patient is using a closed-loop insulin pump it must be used in manual mode for the duration of the test.

Screening procedures include:

- Informed consent obtained or, for subjects aged 12-<18, assent and parental consent.
- Review of inclusion/exclusion criteria
- Review of medical history and diabetes history

- Physical examination
- Vital signs
- Height and weight
- Fundoscopic photography examination conducted by trained personnel and read by an ophthalmologist or optometrist who will provide a signed report.
- Collection of samples for laboratory assessments, including chemistry and hematology safety labs as described in Section 10.8 (including lipids, TSH, T1D antibody measurements, T-cell response, and HbA1c)
- Collection of sample for urinalysis, including protein screen
- If a urinary screen for protein is positive, a 24-hour urine collection for protein will be obtained
- Urine pregnancy test (UPT) (as required for women of childbearing potential [WOCBP])
- Instruction for maintaining glucose control
- Initiation and instructions of unblinded CGM that will continue through Week 52
- Review of insulin use and recent history of hypoglycemic events
- Review and documentation of other concomitant medications used in past 3 months
- Provision of subject diary and review of use
- Baseline HbA1c
- Fasting or non-fasting C-peptide on the initial screening visit if MMTT is not performed.
- Screening MMTT (results required for randomization)

10.1.2 Randomization

Subjects should be randomized no sooner than 6 weeks after diagnosis, unless blood glucose is adequately controlled defined as time in glycemic range (70-180 mg/dL) > 55% by CGM recording over 3 or more days within 5 days prior to screening MMTT. The Randomization Visit must be at least 3 days after the screening MMTT and no longer than 4 weeks after the screening MMTT.

The following procedures will be conducted:

- Review of inclusion/exclusion criteria
- Review and record any changes in medical history

- Review of laboratory results, including T1D antibodies and C-peptide results from screening MMTT
- Review of AEs
- UPT (prior to randomization, as required for WOCBP)
- Vital signs and weight (for adolescent subjects 12-<18 years of age, height)
- Review of subject diary
- Review of CGM use and insulin recording
- Review of concomitant medication use
- Randomization
- Administration of study drug
- Assessment of injection site

10.1.3 Weeks 1, 2, and 4 and Week 3 for subjects aged 14-<18 for the first 6 subjects in cohort B

- Vital signs and weight
- Review of subject diary
- Review of AEs
- Review of concomitant medication use
- Review of previously used injection site
- UPT (Week 4 only, as required for WOCBP)
- Review of CGM use and insulin recording
- Administration of study drug
- Assessment of injection site
- Provision of instructions for at-home administration of study drug
- Provision of study drug and supplies for at-home use (Week 2 [if applicable] and 4 only)
- Collection and documentation of used drug vials (Week 4 only)

10.1.4 Weeks 8, 16, 20, 28, 32, 40, 44, and 48

- Vital signs and weight
- Review of subject diary

- Review of AEs
- Review of concomitant medication use
- Review of previously used injection sites
- UPT (as required for WOCBP)
- Collect sample for HbA1c (Week 16 only as part of MMTT)
- 4-hour MMTT (Week 16 only)
- Review of CGM use and insulin recording
- Collection and documentation of used study drug vials
- Provision of study drug and supplies for at-home use

10.1.5 Weeks 12, 24, and 36

- Vital signs and weight (For adolescent subjects aged 12-<18, height)
- Brief physical examination
- Review of subject diary
- Review of AEs
- Review of concomitant medication use
- Collect sample for T1D antibody assay (Week 12 only)
- Collect sample for $CD8^+$ T-cell assay
- Collect samples for chemistry and hematology safety labs (Weeks 12 and 24 only)
- Collect sample for HbA1c
- Review of CGM use and insulin recording
- 4-hour MMTT (Weeks 12 and 24 only). (Patients using closed-loop insulin pumps must place them in manual mode for the duration of the test)
- UPT (as required for WOCBP)
- Collection and documentation of used study drug vials
- Provision of study drug and supplies for at-home use
- Review of previously used injection sites

10.1.6 Week 52

- If CGM recordings are inadequate during week 50-52, CGM may be continued until the Week 54 visit

- Brief physical examination
- Vital signs and weight and height for adolescent subjects aged 12-<18.
- Review of subject diary
- Review AEs
- Review of concomitant medication use
- Review of previously used injection sites
- Laboratory assessments as described in Section 10.8 (including chemistry and hematology safety labs, T1D antibody measurements, T-cell response
- Urinalysis
- UPT (as required for WOCBP)
- Collect sample for HbA1c as part of MMTT
- 4-hour MMTT. (Patients using closed-loop insulin pumps must place them in manual mode for the duration of the test)
- Collection and documentation of used study drug vials

10.1.7 Week 54: Post-Double-Blind Study Follow-Up

Follow-up safety visit will include a study site visit at Week 54 (+/- 1 week) after the last study visit, including any early termination visits.

The following information will be collected:

- Complete physical examination
- Vital signs and weight
- Review AEs
- Review concomitant medication use
- Chemistry and hematology laboratory assessments, as described in Section 10.8
- Urinalysis
- 24-hour urine collection for protein and creatinine will be obtained if a screening collection was required
- Fundoscopic photography examination will be conducted by trained personnel and read by an ophthalmologist or optometrist who will provide a signed report

10.1.8 2-Year Follow-Up Period (Months 18, 24, 36)

During the 2-year follow-up period after completion of the 52-week double-blind study visits subjects will be seen at 18, 24, and 36 months from first dose with the following assessments:

- Vital signs and weight (and height if under 18 years)
- Physical examination
- Review of AEs
- Review of concomitant medication use including insulin requirements during the preceding 6-week period.
- Collect samples for chemistry and hematology safety labs and HbA1c
- Urinalysis
- 4-hour MMTT. (Patients using closed-loop insulin pumps must place them in manual mode for the duration of the test)
- Fundoscopic photography examination will be conducted at months 24 and 36 by trained personnel and read by an ophthalmologist or optometrist who will provide a signed report.

10.2 Randomization Procedures

Study staff will review results of all screening procedures and the inclusion and exclusion criteria to ensure that the subject qualifies for study participation. Subjects will be randomly assigned to treatment with TOL-3021 or placebo in a 2:1 fashion via Advantage eClinical, Advantage eClinical. Designated staff will log into Advantage eClinical and complete the requested information. The system will specify the coded study drug to be assigned to the subject. Site staff will retrieve the designated study drug vial number to the subject and will record it in source documents. Study drug will be administered to the subject per instructions.

10.3 Study Drug Administration

Study drug will be administered at the site by designated site staff at randomization and Weeks 1, 2, and 4 visits. The first 6 subjects in Cohort B will also have a Week 3 visit and will have their study drug injection administered at that visit. A vial of study drug and injection supplies will be dispensed at Week 2, Week 4, and each monthly visit after Week 4 for administration of the injections at home. Storage requirements will be discussed in detail with each subject or caregiver. Site staff will provide training on the procedures for administration of IM injections. Training materials will be provided to each subject and,

when applicable, caregiver. Study drug will be injected into a large muscle using standard procedures for IM injections.

10.4 Observations and Measurements

10.4.1 Physical Examination

A complete physical examination will be performed at screening and week 54, including examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, and throat (ENT), cardiovascular system (CVS), respiratory system (RS), gastrointestinal system (GI), lymph nodes, musculoskeletal system, and central nervous system (CNS). A detailed medical history must be obtained at the time of screening. A brief physical exam should be done at Week 12, 24, 36 and 52 visits. The brief physical is defined as palpation/percussion/auscultation of the chest, abdomen and extremities, and follow-up of any interim complaints of specific adverse event that require examination/verification.

10.4.2 Fundus Photography

Standard view (not wide field) fundus photography or equivalent procedure is required during screening, Week 54 and Months 24 and 36. The report must be signed by an optometrist or an ophthalmologist. If a subject has had fundus photography completed within the 12 months prior to screening and can provide a signed report, the exam will not have to be repeated at screening.

10.4.3 Vital Signs

Vital signs will include body temperature (°C), respiratory rate, sitting radial pulse rates, and sitting systolic and diastolic blood pressures. Sitting recordings are to be made after the subject has been sitting up for 3 minutes or more. Weight will be recorded at each visit. Height will be recorded at screening, and at randomization, weeks 12, 24, 36, and 52 for subjects aged 12- <18.

10.4.4 24-Hour Urine Protein and Creatinine

If urinary protein screen is positive during the screening period, a 24-hour urine sample will be collected during the screening period and at Week 54 for analysis of urinary protein and creatinine. The 24-hour collection will be returned to the study site. The site will measure and record the total volume and prepare an aliquot to be sent to the central laboratory for assay.

10.4.5 4-Hour Mixed Meal Tolerance Test

Subjects must be fasted for at least 10, and not more than 16, hours prior to the conduct of the MMTT. The 4-hour MMTT will be conducted at the study site with the mixed meal administered between 7:00 AM and 10:00 AM (+ 1 hour), because blood glucose levels will most likely be in the target range of 70-200 mg/dL during those hours. Rapid insulin may be administered 2 hours or short-acting insulin may be administered 6 hours, prior to the MMTT to attain the required glucose levels. Patients using closed-loop insulin pumps must place them in manual mode for the duration of the test.

While the subject is resting comfortably, an intravenous (IV) catheter will be inserted for blood sample collection. The MMTT meal provided by the Sponsor will consist of Boost High Protein Nutritional Energy Drink® (Nestlé) 6 mL/kg body weight, with a maximum of 360 mL consumed within 5 minutes. Blood samples will be collected for assay of C-peptide, glucose, and HbA1c at 10 minutes prior to Boost consumption. Immediately prior to Boost consumption (time=0) and at 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes (± 5 minutes for each timepoint through 60 minutes; ± 10 minutes for each timepoint after 60 minutes) following ingestion of the meal, blood samples will be collected for C-peptide and glucose analysis. Refer to the Laboratory Manual for instructions for collecting and processing glucose and C-peptide samples. Following completion of the MMTT, glucose levels will be checked to determine if insulin should be given before the subject is released. See Manual of Procedures (MOP) for complete instructions for conducting the 4-hour MMTT.

10.4.6 Glucose Monitoring

Rates of serious and clinically important hypoglycemia comprise key secondary efficacy outcomes in this study. Hypoglycemia symptoms will be recorded in the subject diary. For purposes of study outcomes, hypoglycemia is defined as follows:

- Clinically significant hypoglycemia: measured glucose value of <54 mg/dL (3.0 mM/L) by SMBG, CGM, or laboratory measurement
- Severe hypoglycemia (SH): an event requiring assistance of another to actively administer carbohydrate, glucagon, or other resuscitative actions
- Other measures of hypoglycemia:
    - Documented symptomatic hypoglycemia: an event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration <70 mg/dL (3.9 mmol/L)

- Asymptomatic hypoglycemia: an event not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration <70 mg/dL (3.9 mmol/L)
- Nocturnal hypoglycemia: severe or documented symptomatic episodes (as defined above) occurring after the subject has retired for the primary sleeping period

Continuous Glucose Monitoring (CGM)

Unblinded CGM will be used throughout the 52-week study, beginning at screening. Data from CGM will also be required to ascertain that subjects meet the second part of inclusion criterion 3: *time in glycemic range (70-180 mg/dL) >55% by CGM recording over 3 or more consecutive or non-consecutive days.* The sponsor relies on IQVIA and Glooko™ to manage the CGM process from supply of Dexcom G6 CGM units and supplies through data collection. The sponsor has prepared an *"Investigator Manual for Dexcom G6 Continuous Glucose Monitoring (CGM)"* that has been provided as a separate document. This Investigator Manual includes the instructions for the use of CGM devices and the processes followed during a CGM study. It is essential that study staff working with the CGM equipment carefully review and closely follow the Investigator Manual. Additional CGM information is provided in the Manual of Procedures (MOP). If CGM recordings are inadequate during week 50-52, CGM may be continued until the Week 54 visit.

10.4.7 Insulin Requirements

Total daily insulin dose is an important secondary efficacy outcome and must be recorded, regardless of how insulin dose data is collected. Insulin use data is collected by manually entering the amount of insulin administered into the Dexcom receiver or, with Glooko, into the Glooko mobile app. Alternatively, the insulin use data may be collected from an insulin pump or from an InPen™ if the subject is using either of these devices. Any gaps in data may be supplemented by e-CRF data entries.

10.5 Informed Consent, Subject Assent and Authorization to Release Health Information

Written informed consent and subject assent (for subjects 12-<18) will be obtained from all subjects before any study-related procedures (including any screening procedures) are performed. The PI (or designee) may discuss the study and the possibility for entry with a potential subject without first obtaining consent. However, a subject wishing to participate must give written informed consent or assent and parental consent (if applicable) prior to any study-related procedures being conducted, including those performed solely for the purpose of determining eligibility for study participation, and including withdrawal from current medication as may be required prior to study entry. The PI has both the ethical and

legal responsibility to ensure that each subject being considered for inclusion in this study will be given a full explanation of the procedures and expectations for study participation.

Each subject or minor subject's guardian will sign the consent form that has been approved by the same IRB that was responsible for protocol approval for the site. The minor subjects will sign the assent form that has been approved by the IRB. Each informed consent document must adhere to the ethical principles stated in the Declaration of Helsinki (APPENDIX 3) and will include the elements required by FDA regulations in 21 CFR Part 50, as well as the elements required by the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (GCP) guideline and applicable federal and local regulatory requirements. The consent form must include a statement that the Sponsor, their designees, and auditing regulatory agencies will have direct access to the subject's records and medical history for study-related purposes and may use the aggregated and deidentified study results for regulatory approval and drug commercialization purposes after the completion of the study. The informed consent form must be approved by Sponsor in advance of IRB submission.

Once the appropriate essential information has been provided to the subject and fully explained by the PI (or a qualified designee) and it is felt that the subject understands the implications and risks of participating in the study, the IRB/Independent Ethics Committee (IEC) approved consent document shall be signed and dated by both the subject or minor guardian, the person obtaining consent (PI or designee), and by any other parties required by the IRB or other regulatory authorities. If subject is a minor, the IRB/IEC approved assent document shall be signed and dated by both the minor subject and the person obtaining assent (PI or designee). The subject will be given a copy of the signed informed consent document and assent form (if applicable), with the originals kept on file by the PI. All of the above activities must be completed before any study-related procedures, including screening activities, are conducted.

10.6 Instructions to Subjects

10.6.1 Glycemic Control to Maintain HbA1c of 7.0%

At the screening visit, subjects will be given instruction consistent with ADA recommendations on management of their diabetes with the goal of maintaining HbA1c. The HbA1c goal for all subjects is 7.0%.[2] Pre-prandial plasma glucose levels should be in the range of 80-130 mg/dL (4.4-7.2 mmol/L). Peak post-prandial levels should be <180 mg/dL (<10.0 mmol/L). Bedtime levels should be 110-150 mg/dL (6.1-8.3 mmol/L).

[2] ADA recommends a HbA1c target of 7.0 for adults and 7.5 for children and adolescents. In this closely monitored trial, a HbA1c target of 7.0 for children and adolescents is considered to have a positive benefit to risk relationship for younger subjects.

Subjects will be instructed to use injections of short- or long-acting insulin to maintain the target glucose levels. A subcutaneous insulin pump may also be used. Glucose levels should be checked multiple times per day, including in the morning (fasting) and at bedtime. Site staff will review glucose levels, insulin use, and episodes of hypoglycemia at each visit and will provide instruction on any recommended changes to the regimen.

10.6.2 Mixed Meal Tolerance Test

Subjects will be given written instructions on how to prepare for the MMTT with the following main points included:

- The test must be started before 10:00 AM (+ one hour).
- Prior to the MMTT, regular carbohydrate intake should not be modified.
- On the night before the MMTT, subjects must begin a fast of at least 10 hours, but not more than 16 hours, before the scheduled start of the test. Only water may be consumed during the fast.
- The target glucose level at the start of the test is between 70-200 mg/dL (a window of -10% is permitted). The PI (or designee) will provide the subject with guidance on how to achieve this.

Detailed instructions are described in the MOP and will be provided to the subjects.

10.6.3 Subject Diary

Subjects will record information in an electronic subject diary from the screening visit through completion of the study regarding study drug administration and episodes of hypoglycemia. Additional information about the subject diary is included in the MOP and CRF Completion Guidelines.

10.6.4 Study Drug Administration at Home

Subjects and/or caregivers will be provided with instruction and training on the procedures to be used to administer study drug at home. When study drug is dispensed for home administration, syringes, needles, alcohol wipes, and band-aids will be included. Written instructions detailing the procedure for storage of study drug, preparation of study drug for injection, and the procedure to be followed for administering the IM injection will be provided to subjects and/or caregivers. To ensure dosing compliance and good injection technique, subjects and their caregivers will be asked to provide documentation of each injection. The date and site of study drug administration will be recorded in the subject diary.

10.7 End-of-Treatment Procedures

The final study visit of the double-blind treatment period will be at Week 52, or earlier if a subject discontinues the trial before completion of all visits. Procedures to be conducted at the final visit are listed in Section 10.1.6. Any clinically significant abnormal test results (e.g., laboratory findings) seen at the final visit should be followed to resolution or until determined by the PI to be stabilized. Any ongoing SAEs should be followed until the events are resolved, stabilized, or the subject is lost to follow-up. Follow-up safety visits will be conducted for all subjects at Week 54. The Week 54 visit may also be used for collecting CGM if the CGM data collection during weeks 50-52 is inadequate.

10.8 Clinical Laboratory Procedures

Laboratory samples will be analyzed by a central laboratory to ensure consistent interpretation of results. The following laboratory procedures will be conducted at visits as specified below:

Procedure	**Visits**
Chemistry: Electrolytes (sodium, potassium, chloride, bicarbonate), BUN, creatinine, glucose, albumin, calcium, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, direct bilirubin, total protein, creatine kinase, glucose	Screening, Week 12, Week 24, Week 52 (or final visit), Week 54, Month 18, Month 24, Month 36
Chemistry – Lipids: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (samples should be collected in a fasted state)	Screening, Week 12, Week 24, Week 52 (or final visit), Week 54
Hematology: CBC with differential, platelet count	Screening, Week 12, Week 24, Week 52 (or final visit), Week 54, Month 18, Month 24, Month 36
TSH	Screening
Serology: HIV, Hepatitis B, Hepatitis C	Screening
Urinalysis: chemistry and microscopic	Screening, Week 52 (or final visit), Week 54,

Procedure	Visits
	Month 18, Month 24, Month 36
Urine pregnancy test (for WOCBP)	Screening, Randomization, and at each visit (excluding Weeks 1, 2, and 3) through Week 52 (or final visit)
24-hour urine protein and creatinine if urinary protein screen is positive	Screening, Week 54
Antibodies: GAD65, IA-2, ZnT8, insulin	Screening, Week 12, Week 52 (or final visit)
$CD8^+$ T cells	Screening, Week 12, Week 24, Week 36, Week 52 (or final visit)
HbA1c	Screening (collected with hematology sample), and at specified time during MMTT at Screening, Week 12, Week 16, Week 24, Week 52 (or final visit), Month 18, Month 24, Month 36 A sample will also be collected at Week 36.
C-peptide and glucose	Screening (if MMTT does not occur on same day as initial screening visit) At specified times during each 4-hour MMTT (Screening, Week 12, Week 16, Week 24, Week 52, Month 18, Month 24, Month 36)

10.9 Other Procedures

The unblinded CGM procedure is described in the CGM section of the Manual of Operations (MOP).

11 ADVERSE EVENTS

11.1 Definitions

An **Adverse Event (AE)** (also referred to as an adverse experience) is any untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness, clinically significant abnormal laboratory finding, injury, or accident) that emerges or worsens following administration of the study drug and until the end of study participation. The untoward medical occurrence may not necessarily have a causal relationship to the administration of the investigational product. An AE can therefore be any unfavorable and/or unintended sign (including a clinically significant abnormal laboratory result), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

A **Suspected Adverse Reaction (SAR)** is any adverse event for which there is a reasonable possibility that the study drug caused the event. A reasonable possibility implies that there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction.

An **Adverse Reaction (AR)** is any adverse event **caused by the study drug**.

Serious Events, including SAE, Serious Suspected Adverse Reaction (SUSAR), or Serious Adverse Reaction (SAR), as determined by the PI or the Sponsor, are any event that results in any of the following outcomes:

- Death
- Life-threatening condition (i.e., the subject was, in the opinion of the PI, at immediate risk of death from the event as it occurred. It does not apply to an AE that hypothetically might have caused death if it were more severe)
- Persistent or significant disability/incapacity (i.e., the AE results in a substantial disruption of the subject's ability to carry out normal life functions)
- (Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza-like illness, or accidental trauma (e.g., sprained ankle),

which may interfere or prevent everyday life functions but do not constitute a substantial disruption.)

- In-patient hospitalization or prolongation of existing hospitalization (i.e., the AE required at least a 24-hour in-patient hospitalization or prolonged a hospitalization beyond the expected length of stay. Hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine checkups are not SAEs by this criterion)
- Congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a subject exposed to the molecule or investigational product before conception or during pregnancy)
- Important medical event – does not meet any of the above serious criteria but may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

11.2 Pre-Existing Medical Conditions

A pre-existing medical condition is one that is present prior to the start of the study and is to be reported as part of the subject's medical history. It should be reported as an AE only if the frequency, intensity, or the character of the condition worsens during the study.

11.3 Adverse Event Data Collection

All adverse events that occur from the time of signing the informed consent document will be collected.

11.4 Treatment Emergent Adverse Event

A treatment emergent adverse event (TEAE) is any AE reported after the initiation of study drug administration.

11.5 Unexpected Event

An AE is considered unexpected from a regulatory perspective when it is not listed in the package insert/prescribing information, Investigator's Brochure (IB), or, if none of these is available, the general investigational plan (e.g., study protocol, informed consent) for the study product at the specificity and severity that has been observed. Unexpected is a regulatory term, whereas unanticipated is more clinical and should not be used interchangeably. For example, some AEs can be anticipated to occur as a result of a disease or in an older population (e.g., cancer-related deaths in a cancer trial, strokes or acute myocardial infarctions in an older population). However, for reporting purposes, these

anticipated events are not "expected" because they are not listed in the IB (i.e., the study product is not suspected or known to cause them). The Sponsor or designee is responsible for determining expectedness.

11.6 Adverse Events of Special Interest

Local injection site reactions will be graded according to scale show below.

Local reactions (at the injection site)	Severity grade
Injection Site Pain, Burning, Itching or Tenderness	
Pain, burning, or tenderness, mild in intensity causing no limitation of use of limb	**1 (Mild)**
Pain, burning or tenderness moderate in intensity causing minimal impairment of mobility, mental concentration, or sleep	**2 (Moderate)**
Pain, burning, or tenderness causing significant impairment of mobility, mental concentration or sleep	**3 (Severe)**
Redness (Erythema)	
Longest diameter: 2.5 to < 5 cm	**1 (Mild)**
Longest diameter: ≥ 5 to < 10 cm	**2 (Moderate)**
Longest diameter: ≥ 10 cm OR Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissues)	**3 (Severe)**
Swelling (Induration)	
Longest diameter: 2.5 to < 5 cm	**1 (Mild)**
Longest diameter: ≥ 5 to < 10 cm	**2 (Moderate)**
Longest diameter: ≥ 10 cm OR Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissues)	**3 (Severe)**
Ulceration, Subcutaneous abscess	
Longest diameter: 2.5 to < 5 cm	**1 (Mild)**
Longest diameter: ≥ 5 to < 10 cm	**2 (Moderate)**
Longest diameter: ≥ 10 cm	**3 (Severe)**
Bruising	

Local reactions (at the injection site)	Severity grade
Longest diameter: 2.5 to < 5 cm	**1 (Mild)**
Longest diameter: ≥ 5 to < 10 cm	**2 (Moderate)**
Longest diameter: ≥ 10 cm	**3 (Severe)**
Systemic Reactions	**1 (Mild); 2 (Moderate); 3 (Severe)**
Allergic Reactions	**1 (Mild); 2 (Moderate); 3 (Severe)**

11.7 Vital Signs and Physical Exam Findings and Laboratory Results

Vital signs and physical exam and laboratory findings will be captured on specific eCRFs. Vital signs and physical exam or laboratory results assessed as clinically significant should be entered as an individual AE or documented as part of a broader diagnosis reported as an AE in the Advantage eClinical system.

Abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g., vital signs) that are judged by the PI to be clinically significant will be recorded as AE and as SAE if they meet any seriousness criteria outlined previously.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at screening and significantly worsen following the start of the study will be reported as AE. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the PI as more severe than anticipated for the subject's condition, or that are present or detected at the start of the study but do not subsequently worsen, will not be reported as AE. The PI will exercise medical judgment in deciding whether abnormal laboratory values or other abnormal assessments are clinically significant while also considering the normal range for age and gender, among other factors.

11.8 Guidelines and Evaluation

11.8.1 Assessment and Recording

The PI is responsible for evaluating all AEs and determining the severity of the event. AEs will be collected from the time the subject signs informed consent until the time an event is resolved or until the subject completes or terminates from the study, whichever comes first.

The PI will assess subjects at each scheduled study visit post-treatment for the occurrence of AEs by observation of the subject and questioning the subject in an objective manner. In order to avoid bias in eliciting AEs, subjects should be asked the following non-leading question: *How have you felt since your last visit?* All AEs (serious and non-serious) reported by the subject must be recorded on the source documents and eCRFs. The PI should use the following definitions when assessing severity of an AE:

- Grade 1 = mild AE; subject is aware of symptoms or has minor findings. No interference with activity and no medical intervention/therapy required.
- Grade 2 = moderate AE; some interference with activity and/or minimal medical intervention/therapy required (clinical judgment should be used to determine what is a minimal intervention/therapy; short-term use of acetaminophen for a headache is an example of a minimal therapy).
- Grade 3 = severe AE; interferes significantly daily activity and/or requires more than minimal medical intervention/therapy.

11.8.2 Causality of an Adverse Event

The PI will use the following question when assessing causality of an AE to the study product/intervention: *Is there a reasonable possibility that the study product/intervention caused the event?*

"Reasonable possibility" means that there is evidence to suggest a causal relationship between the study product/intervention and the AE. This does NOT mean that the relationship cannot be ruled out. Examples of types of evidence that would suggest a causal relationship between the study product and AE are the following:

- A single occurrence of an event that is uncommon and known to be strongly associated with study product exposure (e.g., angioedema, hepatic injury, Stevens-Johnson syndrome).
- One or more occurrences of an event that is not commonly associated with study product exposure but is otherwise uncommon in the population exposed to the study product (e.g., tendon rupture).

The PI will use clinical judgment to determine if he or she believes there is a reasonable possibility of a causal relationship with the study product. Using merely the temporal sequence to determine causality is not sufficient. Rather, known or presumed pharmacologic or biologic action of the study product or class of study products should be considered in the assessment. Dose-response relationship, prior similar AE(s) with similar or identical study product(s), improvement following study product discontinuation, and recurrence upon re-challenge are among the most robust supportive evidence for a causal

relationship. Alternative etiologies, such as natural history of underlying diseases, new disease/disorder development, concomitant therapy, and other risk factors, will be considered and investigated. The PI will also consult the IB or other study product information for his/her assessment.

An affirmative answer designates the event as a Suspected Adverse Reaction (SAR).

The following information will be reported for each event and must be reviewed by the PI or designee: 1) description of the event, 2) start date, 3) stop date, 4) severity of the event, 5) causal relationship to study product/intervention, 6) seriousness, 7) outcome of the event, and 8) if action with study product/intervention was required.

11.8.3 Reporting Requirements

11.8.3.1 Adverse Event Reporting

Regardless of severity or relationship to the study product, all AEs occurring after informed consent and through the Month 36 study visit must be recorded in the subject's eCRF. The following information and assessments should be recorded in the AE section of the eCRF:

- The date of onset of the event and date it ended.
- The signs, symptoms, or diagnosis of the event.
- The AE severity using the criteria outlined above.
- The relationship of the event to the study drug.
- The seriousness of the event according to definitions outlined above.
- A description of any action taken regarding study drug disposition.
- Any additional data which might be relevant to the event.

All adverse events, regardless of the PI's determination as related or unrelated to the investigational product, are to be reported in the Advantage eClinical. AEs will be reviewed on a weekly basis by the medical monitor or his/her designee and cumulative AE reports will be shared with the data safety monitoring board per the charter. All SAEs will be reviewed within one business day of notification.

11.8.3.2 Serious Adverse Events Reporting

SAEs are considered immediately reportable events.

Full details of the SAE event, treatment, and an assessment of the relationship to the study drug must be provided in the report. SAEs will be captured from time of informed consent through the Month 36 visit.

If a subject experiences an SAE, the PI must:

- Report the SAE by entering the data into the Advantage eClinical system immediately (within 24 hours) after the PI becomes aware of the event. If Advantage eClinical is not available, the site should report the SAE via email at tol_safety@emmes.com.
- Obtain and maintain all pertinent medical records, information, and medical judgments of medical personnel who assisted in subject's treatment and follow-up and document on CRF as appropriate.
- Provide a more detailed report to both Emmes (via Advantage eClinical) and the IRB/IEC no later than seven days after the PI discovers the event as further information becomes available, and, when necessary, update the information with follow-up information, including outcomes. This report should include a statement on whether the event considered related to the use of investigational product by the PI.
- The PI will notify the IRB/IEC of the SAE according to specific IRB/IEC requirements.

The following attributes must be provided by the PI or designee immediately on being notified of a serious event:

- Description of event, diagnosis when applicable
- Date of onset and resolution (if known when reported)
- PI/designee assignment of severity
- PI/designee assessment of causality to the study product/intervention
- Action taken with study product/intervention (e.g., stoppage)

After the SAE has been reported by the PI and assessed by the Medical Monitor and the Sponsor, they must report the event to the appropriate regulatory authorities using one of two options:

- Standard reporting (report in the annual report). This option applies if the AE is classified as one of the following:
    1. Serious, expected, suspected adverse reaction (serious, expected, and related)
    2. Serious and not suspected adverse reaction (serious and not related)
- Expedited reporting. This option is required if the AE is classified as serious and unexpected, suspected adverse reaction (serious, unexpected, and related).

The Sponsor must report an AE as a suspected adverse reaction (related AE) only if there is evidence to suggest a causal relationship between the study drug and the AE, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with study drug.
- One or more occurrences of an event that is not commonly associated with the study drug but is otherwise uncommon in the population exposed to the study drug.
- Aggregate analysis of specific AEs and SAEs observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of the study drug) that indicates those events occur more frequently in the study drug group than in a concurrent or historical control group.
- Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or *in vitro* testing that would result in a safety-related change in the protocol, informed consent, or other aspects of the overall conduct of the trial.

The Sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor must notify FDA and all participating PIs in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15calendar days after the initial receipt of the information regarding any AE associated with the use of the investigational product that is both serious and unexpected.

All SAEs will also be reported quarterly to the DSMB.

The PI will collect information on SAEs until the subject’s health has returned to baseline status, all parameters have returned to normal, or remaining health issues have otherwise been explained.

11.8.3.3 Collection and Follow-up of AEs and SAEs

Qualified research staff will elicit subject reporting of AEs, including SAEs, from the time the subject signs informed consent. The research staff will obtain as much information as possible about the reportable AE/SAE to complete the AE/SAE CRF(s) and will consult with the PI as warranted. The information will be reviewed by the PI and assessed for seriousness, severity, and causal relationship. Appropriate site staff will review all AE documentation and verify accuracy of assessments to ensure that all AEs are appropriately reported and to identify any unreported AEs that require reporting. Each participating site’s

PI is responsible for study oversight, including ensuring human research subject protection by designating appropriately qualified, trained research staff and assessing, reporting, and monitoring AEs.

CRAs will visit the study sites and review the study data on a regular basis. They will also promptly advise sites to report any previously unreported AEs and ensure that the AEs are being followed appropriately by the research staff. The CRA will ensure that any unreported or unidentified SAEs discovered during visits are promptly reported by the site. Staff education, re-training, or appropriate corrective action plan will be implemented at the participating site when unreported or unidentified AEs are discovered, to ensure future identification and timely reporting by the site. The study Medical Monitor or his/her designee will review all reported AEs/SAEs throughout the study. Multiple safety monitoring layers will be provided by:

- All SAEs reported will undergo Medical Monitor review within one business day of notification of the SAE
- Weekly review of all reported AEs using listings generated from the data system; the review will entail both what was reported in the preceding week and cumulatively from study inception for trends and safety signals
- MedDRA coding of all AEs will be performed on a quarterly basis to ensure consistency of terminology; the coding process will also entail review for trends and safety signals
- Document preparation for periodic DSMB meetings; cumulative safety data will be reviewed for trends and safety signals at the time of document preparation and review

The study DSMB will also review safety data for this trial periodically at regularly scheduled meetings.

AEs, including SAEs, will be reported to the central IRB according to central IRB requirements. This will be reviewed on site by the CRA for the study.

All SAEs as defined earlier will be reported to the PI, Medical Monitor or his/her designee, and the Sponsor.

11.8.3.4 Pregnancy

Pregnancy is not considered an AE, but any pregnancies that occur during the study will be tracked on a pregnancy case report form. Pregnancy is an exclusion criterion. If a study subject was pregnant on the day of study product administration (e.g., due to a false negative pregnancy test) or becomes pregnant afterwards, the subject will be followed through the end of her pregnancy. The subject will not receive any additional doses of

study medication but would be encouraged to continue to attend the remaining study visits. If the subject is unwilling to do so, she should be asked to return to the study center to complete the assessments specified in the End-of-Study Visit (Week 52). Any complications of pregnancy or termination of pregnancy due to medical reasons will be considered a SAE as defined in Section 11.1. The PI should be informed immediately of any pregnancy, and all available pregnancy information should be entered into Advantage eClinical within 24 hours of becoming aware of the event. The PI should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. The site will be asked to follow-up with the subject periodically during the pregnancy for ongoing health and safety information through the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the Advantage eClinical system as it becomes available. If the pregnancy results in normal childbirth with a healthy, normal infant, no SAE will need to be reported. If the pregnancy results in a congenital anomaly/birth defect, an SAE must be reported following the SAE reporting procedures in the protocol. Any premature termination of the pregnancy will be reported.

11.9 Follow-Up of Adverse Events and Serious Adverse Events and Final Reports

AEs, including SAEs, will be followed until resolved or resolved with sequelae, (i.e., medically stable). AEs ongoing at the time of the last visit will be marked as ongoing however SAEs will be followed through resolution or up to 30 days, whichever comes first. The site must actively seek information about an SAE as appropriate until the SAE is resolved or considered resolved with sequelae.

Non-serious AEs that are identified during the last scheduled study visit (Week 54 or early discontinuation) must be recorded on the AE CRF as ongoing.

Any clinically significant abnormal test results (e.g., laboratory findings) at the final assessment should be followed to resolution or until determined by the PI to be stabilized. Repeat tests may be indicated to establish this.

If a subject has any clinically significant, study-related abnormalities at the end of the study, the Medical Monitor should be notified, and every effort should be made by the PI to arrange follow up evaluations at appropriate intervals to document the course of the abnormalities.

All suspected serious adverse reactions regardless of expectedness must be followed until resolution or until the event is considered resolved with sequelae (i.e., medically stable, beyond the end of the study if needed). All other events that cannot be resolved within 30 days after last study contact (e.g., consent withdrawal, lost to follow-up) or after the last

study visit will be considered ongoing and entered into Advantage eClinical with that outcome.

SAEs that are identified on the last scheduled contact must be recorded on the AE CRF page and reported to Emmes according to the reporting procedures outlined in Section 11.8.3 and the Safety Monitoring Plan (SMP). This may include unresolved previously reported SAEs, or new SAEs. The PI should follow these SAEs until the events are resolved, or the subject is lost to follow-up. The PI should continue to report any significant follow-up information to the Medical Monitor and the IRB up to the point where the event has been resolved. Resolution means the subject has returned to the baseline state of health, or the PI does not expect any further improvement or worsening of the subject's condition.

Any new SAEs reported by the subject to the PI that occur after the last scheduled contact and are determined by the PI to be reasonably associated with the investigational product should be reported in Advantage eClinical and to the IRB.

Table 5: Adverse Event Status and Outcomes

Status	Definition
Recovered/ Resolved	One of the possible results of an adverse event outcome that indicates that the event has improved or recuperated.
Recovered/ Resolved with sequelae	One of the possible results of an adverse event outcome that indicates the subject recuperated but retained pathological conditions resulting from the prior disease or injury.
Recovering/ Resolving	One of the possible results of an adverse event outcome that indicates that the event is improving.
Not Recovered/ Not Resolved	One of the possible results of an adverse event outcome that indicates that the event has not improved or recuperated.
Fatal	The termination of life as a result of an adverse event.

12 DATA MANAGEMENT AND PROCEDURES

12.1 Design and Development

This protocol will utilize Emmes, a CRO selected by the Sponsor. Emmes will be responsible for developing the CRFs, developing and validating the clinical study database, ensuring data integrity, and training site staff on applicable data management procedures. A web-based distributed data entry model will be implemented. Advantage eClinical will be developed to ensure that guidelines and regulations surrounding the use of computerized systems used in clinical trials are upheld. The remainder of this section provides an overview of the data management plan associated with this protocol.

12.2 Data Center Responsibilities

Emmes will 1) develop and apply data management procedures to ensure the collection of accurate and good-quality data, 2) provide final CRFs and electronic CRFs (eCRFs) for the collection of all data required by the study, 3) develop data dictionaries for each CRF that will comprehensively define each data element, 4) prepare instructions on the use of Advantage eClinical and the completion of CRFs, 5) conduct ongoing data validation and cleaning activities on study data collected from all study sites; and 6) perform data validation and cleaning activities prior to any interim analyses and prior to the final study database lock.

12.3 Data Acquisition and Entry

Data will be collected at the study sites on source documents and entered by site staff into eCRFs in Advantage eClinical. Emmes will provide the sites with paper CRF source documents and completion instructions. Data will be entered into Advantage eClinical by site staff according to the instructions provided during training and with the guidelines established by Emmes. Data entry into the eCRFs must be performed by authorized individuals.

Subject diary data will be captured electronically and incorporated into the study database for analysis. Laboratory data and CGM data will be entered into an electronic database and transferred to Emmes for inclusion in the study database.

The PI at each site is responsible for maintaining accurate, complete, and up-to-date research records. In addition, the PI is responsible for ensuring the timely completion of eCRFs for each research subject. The PI will be required to provide attestation for each subject that all information entered into Advantage eClinical is correct and accurate.

12.4 Data Validation and Editing

Data will be entered on source document prior to entry in Advantage eClinical. Site staff are expected to follow ALCOA-C (Attributable, Legible, Contemporaneous, Original, Accurate, and Complete Data) principles when completing and editing source documents.

Emmes will monitor eCRFs for completeness and accuracy throughout the study. Dynamic reports listing missing values and forms are available to sites in Advantage eClinical. Emmes also will identify inconsistencies within eCRFs and between eCRFs and post-data clarification requests or queries in Advantage eClinical on a scheduled basis. Emmes will develop a Data Validation Plan detailing the procedures for data collection, edit checks, data queries and corrections, and data processing to provide a clean database for analysis. The Sponsor will approve the plan.

In addition, Emmes will conduct on-site monitoring visits, during which audits comparing source documents to the data entered on the eCRF will be performed. Any discrepancies identified between the source document and the eCRF will be corrected by the site. Sites will resolve data inconsistencies and errors by updating the appropriate source document as well as entering all corrections and changes into Advantage eClinical.

12.5 Data Transfer / Lock

At the conclusion of data collection through 30 patients reaching Week 24, Week 24 and Week 54, Emmes will perform cleaning activities and will “freeze” the study database. A final data “lock” will be conducted upon completion of data collection for the follow-up safety visits through Month 36. Prior to the final lock, Emmes will perform final cleaning activities and the study database will be “locked” from further modification. SAEs will be captured from time of informed consent through the Month 36 visit. The final analysis datasets will be transferred to the Sponsor, as requested, for storage and archiving.

12.6 Data Training

Emmes will train site staff on the assessments, eCRF completion guidelines, data management procedures, and the use of Advantage eClinical.

12.7 Data Quality Assurance

To address the issue of data entry quality, Emmes will follow a standard data monitoring plan. An acceptable quality level prior to study lock or closeout will be established as a part of the data management plan. Data quality summaries will be made available during the protocol.

13 STATISTICAL CONSIDERATIONS

13.1 Sample Size/Power Considerations

Up to 99 subjects will be randomized 2:1 to active and placebo treatments. For analytic purposes all subjects 12-<41 years of age will be considered cohort A. A minimum of 18 and a maximum of 24 subjects aged 12 and <18 years of age will comprise Cohort B. Adult subjects aged 18-<41 will comprise cohort C. The proposed study is powered to detect approximately an overall 13% difference between treatment and placebo groups in a repeated measures mixed model analysis of the mean plasma C-peptide AUC over 4-hour MMTT at Week 12, 16, and 24 weeks as compared to the subject's baseline. A total of 90 subjects will provide 90% power to detect this difference at a significance level (alpha) of 0.05; a total of up to 99 subjects will be randomized to allow for an anticipated drop-out rate of approximately 10%. Insulin secretion as reflected by C-peptide declines more rapidly in younger patients, including adolescents. TOL-3021 is expected to have equal or even greater effect in the adolescent population compared to the effect observed in the phase 2a study of adult subjects only. An effect was detectable in the 17 adults who received 12 weeks of 1.0 mg treatment in that study. Based on assumption of an overall difference between treatment and placebo groups of 20%, then a minimum of 18 adolescents aged 12-<18 (Approximately 12 active and 6 placebo) would provide 80% power to detect a difference at a significance level (alpha) of 0.05, providing an initial estimate of efficacy and a description of safety in this population Cohort B (ages 12-<18) and cohort C (ages 18-<41) and will be analyzed separately as well as combined.

13.2 Interim Data Analysis

When the first 30 treated subjects have completed the Week 12 visit, summary and individual safety data will be submitted to the DSMB for review to support expanding enrollment in TOL-3021 studies to adolescent subjects aged 12.0 to <18 years of age.

An interim analysis will be performed when the first 30 subjects complete 24 weeks of treatment. The DSMB will review data in a blinded manner. Alpha spending will be utilized by the O'Brien-Fleming method, with 0.0052 of the alpha being spent at the interim analysis, saving the remaining 0.0498 alpha for the final analysis. Criteria for the review and any decision will be agreed with the DSMB. A small group of Tolerion management who are not related to the conduct of the trial will be provided unblinded data results from this interim analysis for administrative purposes. This list of individuals to be unblinded will be identified, and the process for these individuals to receive unblinded data results will be finalized prior to the unblinding for this interim.

Unblinded data summaries including safety outcomes and efficacy assessments will be prepared for the sponsor during the study using all data collected through Week 36. A final analysis of the data will be conducted at 52 weeks.

13.3 Data Analysis

Primary endpoint analyses will be conducted after all enrolled participants have completed Week 24. A final analysis of all data will be conducted after Week 52 is complete. Safety data and efficacy analyses will be updated at Week 36.

13.3.1 Analysis of Baseline and Demographic Data

Demographics and baseline characteristics will be summarized by treatment group for all randomized subjects. Data will be pooled for all centers for baseline data analysis. The F test of the Type III treatment factor from a one-way ANOVA model including only the treatment factor will be used to analyze numeric variables. The Fisher's Exact test will be used to analyze categorical data. Overall comparisons between treatment groups will be performed. Nominal comparisons between treatment groups will be performed for baseline data to assess treatment group balance across the various baseline characteristics. These comparisons will be considered non-inferential; nominal p-values will be reported with no statistical conclusion and no adjustment for multiple tests will be made.

13.3.2 Safety Analysis

All randomized subjects who received treatment will be included in a final safety data analysis which will include all data through Week 52 as the as-treated cohorts. In addition to descriptive summaries of all AEs by treatment group, Fisher's Exact test will be used to compare the incidence of the most frequent AEs or SAEs between treatment groups. The MedDRA system will be used to map each AE verbatim term to the system organ class (SOC) and preferred term (PT) for summary purposes. AE data, including the AE verbatim term and the associated AE preferred term, will be provided in the subject data listings. Note that once enrollment of TOL-3021 studies is expanded to adolescent participants aged 12.0 to <18 years of age, data from adolescent participants will be summarized and presented separately from data on adult participants in all subsequent DSMB review meetings.

Adverse event incidence summaries, including incidence rates of SAEs and TEAEs, will be generated on a monthly basis on live data. These will be reviewed in real time by the Sponsor and Emmes, with the treatment groups labeled as "A" and "B." An excessive rate in any specific AE or SAE may result in referral to the DSMB for review. The study statistician will maintain these reports and all resulting correspondence. The code may be requested to be broken by the DSMB after receiving Sponsor approval.

Other safety data, such as laboratory data, physical examination data, and abnormal findings, will be tabulated and/or graphically displayed. Concomitant medications used during the study will be summarized. Medications discontinued prior to the randomization date will be excluded from the summary.

Safety data collected after Week 52 will be analyzed separately from safety data collected during the controlled trial period.

After 6 subjects aged 14-<18 years of age have been enrolled with the last subject having a minimum of 2 injections with at least 1 week follow-up after the 2nd injection. Safety data from these initial subjects will be evaluated before opening the study to subjects 12 and older.

13.3.3 Efficacy Analysis

The analysis of the primary outcome, MMTT-stimulated 4-hour mean C-peptide AUC at multiple time points will test the null hypothesis of "no treatment group difference" versus the two-sided alternative using the intent-to-treat (ITT) cohorts. The treatment-group main effect hypothesis test will be derived from a repeated measures mixed model of the log-transformed 4-hour C-peptide AUC response at 12, 16 and 24 weeks, adjusted for the baseline C-peptide AUC, age group and T1D-duration group. Missing data for this analysis will be imputed for each subject based on any available data. To be imputed, a data point must have data for the visit before and the visit after the missing data point, and the slope between the two non-missing visits will be used to impute the missing data. A secondary method using a mixed model for repeated measures (MMRM) approach will also be utilized for the primary and secondary endpoints that cannot be imputed using the approach above. Additional details for this approach will be provided in the statistical analysis plan.

13.3.4 Pharmacokinetic / Pharmacodynamic Analysis

No pharmacokinetic or pharmacodynamic analyses will be conducted.

13.3.5 Immunoprofiling

Quantum dot (Q-dot) responses within the qualifying subpopulation (HLA class I types for which multimers are available) will be conducted to confirm induction of specific autoantigen tolerance. Analysis of immunological endpoints will be conducted on the intent-to-treat (ITT) and per protocol populations. Pre-treatment and post-treatment values for T cell responses and T1D antibodies will be summarized over time, as well as the frequency of T cell immune responses and T1D antibodies and the strength of the immune responses.

Q-dot (quantum dot) flow cytometry will be used to measure $CD8^+$ T cell responses to various epitopes from individuals who have HLA-A2, HLA-A3, or HLA-B7 haplotypes.

The frequency of $CD8^+$ T cells responding to proinsulin GAD, IA2, PPI leader sequence, islet amyloid polypeptide (IAPP), and IGRP before and after the 52-week treatment period will be measured and compared to C-peptide response.

It is known that immune responses to injected insulin may develop after initiation of insulin therapy. Consequently, antigen presentation resulting from insulin therapy may act as to confound immune responses induced by TOL-3021. This interaction will be accessed by measuring $CD8^+$ T cell responses to an insulin epitope (insulin B10-18) contained within injected human insulin and to two other epitopes unique to TOL-3021. $CD8^+$ responses to viral epitopes on EBV, CMV, and measles will also be measured with Q-dot flow cytometry.

Serum insulin, glutamic acid decarboxylase, IA-2 and ZnT8 autoantibodies, and insulin autoantibody affinity will be measured before, during, and after the 52-week treatment period. Insulin autoantibody isotypes (IgA and IgM) and IgG subclasses for subjects will also be evaluated.

14 ESTIMATED DURATION OF THE STUDY

This study has an estimated duration of up to 4 weeks for screening, 52 weeks of treatment and two years of follow-up after randomization per subject. This study has an estimated overall duration of approximately 42 months, including recruitment, screening, a 52-week treatment, and a 2-year safety follow-up.

15 ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

15.1 Medical Care

Subjects will receive routine care throughout the trial. The study is designed to monitor the status of the subject's T1D throughout the study by collecting information on daily insulin use, glucose levels, episodes of hypoglycemia, local and systemic reactions to study drug, AEs, and use of concomitant medications. Subjects will be provided with information for management of their diabetes at the screening visit and throughout the study. Subjects will have clinic visits weekly during the 4 weeks after randomization and then approximately monthly until study completion.

15.2 Subject Information and Informed Consent

The informed consent process (and assent process for 12-<18 years of age) is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. The consent and assent forms will be submitted with the protocol for review and approval by the IRB and sponsor prior to use. The subject and

subject's guardian where applicable, will be asked to read and review the document. The PI or designee will explain the research study to the subject and answer any questions that may arise. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects (and parent/legal guardian where applicable) will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The subject (or legally accepted surrogate) will sign the informed consent/assent document prior to any procedures being done specifically for the study. Potential subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent and assent document, where applicable will be given to the subject for their records. The informed consent process will be documented in the source document, and the form signed, before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.3 Ethics Committee Review

The protocol, informed consent and assent form and any subject information sheet must be approved in writing by the appropriate Department of Health and Human Services (HHS)-registered IRB before the study can be initiated at a site. A copy of the IRB approval must be sent to the Sponsor (or designee) along with a list of the IRB members and their occupations/affiliations. IRB approval is also required for any advertising or other material used for subject recruitment, and for instructions provided to the subject. If the protocol is amended, the PI must sign the revised protocol and submit the amendment to the IRB for review and approval prior to implementation of the changes specified in the amendment. The PI must report promptly to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others, including all serious adverse events that have resulted in an expedited safety report to the FDA. No drug will be shipped to a site until IRB approval has been granted and the Sponsor or designee has been notified of this in writing.

The PI is responsible for obtaining continued review of the clinical study at intervals not to exceed one year or otherwise specified by the IRB. The PI must provide the Sponsor (or designee) with written documentation of the continued review.

15.4 Standards

15.4.1 Ethics and Responsibility

This study will be conducted in compliance with the protocol and according to US and international standards of Good Clinical Practice (FDA regulations 21 CFR 312 for IND studies and ICH guidance E6) for all studies. PIs must submit all essential regulatory documentation, as required by local and national regulations (including IRB approval of the protocol and informed consent form) to the Sponsor or its designee before investigational product will be shipped to the study site.

15.4.2 Monitoring, Compliance, Quality

All aspects of the study will be monitored by the authorized representatives of the Sponsor according to GCP and Standard Operating Procedures (SOPs) for compliance with applicable government regulations, (i.e., Informed Consent Regulations and IRB regulations).

The PI must conduct the protocol in compliance with applicable GCP regulations and guidelines, applicable informed consent regulations, and the Declaration of Helsinki (APPENDIX 3). Every attempt must be made to follow the protocol and to obtain and record all data requested for each subject at the specified times. If data is not recorded per protocol, the reasons must be clearly documented on the source documents or other study records.

Access to all records, both during the trial and after trial completion, should be made available to the Sponsor or its designee at any time for review and audit to ensure the integrity of the data. The PI must notify the Sponsor or its designee immediately if the responsible IRB/IEC has been disqualified by local health authorities or if proceedings leading to disqualification have begun.

Before study initiation, at a site initiation visit or at a meeting with the PI(s), a Sponsor representative will review the protocol and study documents, including eCRFs, with the PI(s) and their staff.

CRAs will perform ongoing site visits to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents. In addition, the CRA will review the site's adherence to the protocol and to GCP, as well as the progress of enrollment. The CRA will ensure that consent is being sought and obtained in compliance with applicable regulations, and that the investigational product is being stored, dispensed, and accounted for according to

specifications. The PI or designee must promptly complete the eCRFs after the subject's visit.

Source documents are where subject data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory results, memoranda, evaluation checklists, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, photographic negatives, magnetic media, and medico-technical departments involved in the clinical trial.

To facilitate monitoring, the PI(s) and institution(s) must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB review. The site must also allow inspection by applicable regulatory authorities. The PI and key trial personnel must be available to assist the monitor during these visits.

When clinical observations are entered directly into the site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with FDA requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system (for clinical research purposes) would be one that (1) allows data entry only by authorized individuals, (2) prevents the deletion or alteration of previously entered data and provides an audit trail for such data changes (e.g., modifications of file), (3) protects the database from tampering, and (4) ensures data preservation.

If a site's computerized medical record system is not adequately validated for the purposes of clinical research (as opposed to general clinical practice), applicable hardcopy source documents must be maintained to ensure that critical protocol data entered into the eCRFs can be verified.

Subjects (or their legal representatives) must also be allowed access to their medical records. Subjects will be informed of the importance of increased record access and granted permission by signature on the informed consent document prior to Screening. No information in these records about the identity of the subjects will leave the study center. Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of primary efficacy and safety variables. Additional checks of the consistency of the source data with the CRFs will be performed according to the study-specific monitoring plan.

Emmes, the CRO monitoring this clinical trial, will follow standard operating procedures for monitoring this study in accordance with GCP recommendations and FDA regulatory requirements. Any site not meeting the minimum requirements to initiate the trial, or that

has administrative, procedural, or data quality deficiencies that require correction in order to comply with or meet regulatory requirements or the protocol, will be notified in writing of the deficiencies and permitted a reasonable opportunity to rectify deficient conditions. After study initiation, the inability of the site to rectify seriously deficient conditions in a timely manner or to maintain compliance with regulatory requirements may be cause for termination of study activities, closure of the investigational site, and notification of that decision to the relevant IRB) or IEC and other regulatory authorities as appropriate.

Participating sites will have an initial monitoring visit, routine interim monitoring visits during the study, and a study close-out visit conducted by experienced CRAs. Additional monitoring visits may be performed for cause or if the volume of information to be reviewed cannot be easily completed in a single visit. All attempts will be made to schedule the study visits in advance so that necessary site staff and appropriate records will be available during the monitoring visit.

Each monitoring visit will utilize a standardized checklist of elements to be reviewed at the site, tailored to the specific requirements of this study. Site monitoring visits will routinely review the participating site staff roster; administrative and financial documents; required regulatory documentation; status of IRB/IEC approvals; changes or actions taken since any previous visit; subject recruitment status, screening, enrollment, and follow-up visit records; documentation of informed consent for each subject; review of adverse events; investigational product storage conditions; outstanding data clarifications, and data elements against source documentation. Site visits will follow the study-specific monitoring plan procedures, and a summary report will be prepared for study records. The CRA will also investigate any questions concerning adherence to regulatory requirements. Any administrative concerns will be clarified and followed. The CRA will maintain contact with the site through frequent direct communications with the study site by e-mail and telephone. The PI and all other site personnel agree to cooperate fully with the monitor and will work in good faith with the CRA to resolve any and all questions raised, and difficulties detected by the CRA.

15.4.3 Quality Assurance Audits and Quality Control

In addition to the routine monitoring procedures, audits of clinical research activities in accordance with Standard Operating Procedures (SOPs) may be performed to evaluate compliance with the principles of GCP. A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If a regulatory authority requests an inspection, the PI must inform the Sponsor immediately that this request has been made.

Study conduct may be assessed during the course of the study by a quality assurance/compliance representative(s) to ensure that the study is conducted in compliance

with the protocol and GCP. They will be permitted to inspect the study documents (study protocol, CRFs, investigational product, original study-relevant medical records). All subject data will be treated confidentially.

15.5 Confidentiality

All information generated in this study must be considered highly confidential and must not be disclosed to any persons not directly concerned with the study without written prior permission from the Sponsor. Authorized regulatory officials and Sponsor personnel or their representatives will be allowed full access to inspect and copy the records. All investigational products, subject bodily fluids, and/or other materials collected shall be used by site and its personnel solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor.

A subject's privacy and confidentiality will be respected throughout the study. Each subject will be assigned a sequential identification number. This number and subject initials, rather than the subject's name, will be used to collect, store, and report subject information.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records and the informed consent document signed by the subject must permit such access.

The study subject's contact information will be securely stored at each clinical site for internal use during the study.

US FDA regulations (21 CFR 312.62[c]) and ICH Guideline for GCP (see Section 4.9 of the guideline) require that records and documents pertaining to the conduct of this study, including eCRFs and consent forms, must be retained by the PI for two years after the last marketing application approval in an ICH region or after at least two years have elapsed since formal discontinuation of clinical development of the investigational product. All state and local laws for retention of records also apply.

No records should be disposed of without the written approval of the Sponsor or its designee. Written notification should be provided to the Sponsor or its designee for transfer of any records to another party or transfer to another location.

Study subject research data will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a

unique study identification number. The study data entry and study management systems used by clinical sites and Emmes research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived as specified by the Sponsor.

The confidentiality terms set forth in this protocol are in addition to, and not in lieu of, those set forth in the Clinical Trial agreement between the Sponsor and the site. In the event of any conflict between this Section 15.5 of the protocol and the applicable section of such Clinical Trial Agreement, such section of the Clinical Trial Agreement will govern.

15.6 Protocol Amendments

Protocol modifications, except those intended to reduce immediate risk to study subjects, may only be made by the Sponsor. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB is notified within five days.

Any permanent change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB and the PI must await approval before implementing the changes. the Sponsor or their designee will submit protocol amendments to the appropriate regulatory authorities for approval.

If in the judgment of the IRB, the PI, and/or the Sponsor, the amendment to the protocol substantially changes the study design and/or increases the potential risk to the subjects and/or has an impact on the subjects' involvement in the trial, the currently approved written informed consent form will require similar modification. In such cases, informed consent will be renewed for subjects enrolled in the study and signed before continued participation.

15.7 Protocol Adherence

Site staff will be thoroughly trained on all study procedures prior to site initiation. Protocol adherence will be monitored throughout the study. Any protocol deviations will be recorded by the study monitor and the Sponsor will be notified. Protocol deviations will be reported in the Clinical Study Report.

16 USE OF INFORMATION

The information generated in this study will be used by the Sponsor in connection with the development of the product and therefore may be disclosed to government agencies in various countries. To allow for the use of information derived from the study, it is

understood that the PI is obliged to provide the Sponsor with complete test results, all study data, and access to all study records.

Any results of medical investigations with the Sponsor products and/or publication/lecture/ manuscripts based thereon, shall be exchanged and discussed by the PI and Sponsor representative(s) in accordance with the terms of the Clinical Trial Agreement. Due regard shall be given to the Sponsor's legitimate interests (e.g., manuscript authorship, obtaining optimal patent protection, coordinating and maintaining the proprietary nature of submissions to health authorities, coordinating with other ongoing studies in the same field, and protecting confidential data and information). Site may only publish such information if permitted under the terms of the Clinical Trial Agreement, and then only in accordance with the terms thereof.

In cases of publications or presentations of material arising from multicenter clinical investigations, The Sponsor will serve as coordinator and referee. Individual PIs, who are part of a multicenter investigation may not publish or present data that are considered common to a multicenter investigation without the consent of the other participating PIs and the prior review of the Sponsor.

In case of disagreement amongst the PIs participating in a multicenter investigation, the Sponsor will be the final arbiter. Comments shall be given without undue delay. If they are not accepted, the senior author of the manuscript and representatives of the Sponsor shall promptly meet to discuss further and endeavour to agree mutually on the final wording and/or disposition of the publication. The above procedure also applies to information on prematurely discontinued and other non-completed studies.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as outlined previously. The Sponsor will not quote from publications by PIs in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference).

The publication / use of information terms set forth in this protocol are in addition to, and not in lieu of, those set forth in the Clinical Trial agreement between the Sponsor and the site. In the event of any conflict between this Section 16 of the protocol and the applicable section of such Clinical Trial Agreement, such section of the Clinical Trial Agreement will govern.

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18 APPENDICES

APPENDIX 1: SCHEDULE OF ASSESSMENTS DURING 52-WEEK BLINDED STUDY

	Screening	Screening MMTT	*Randomization	Week 1	Week 2	Week 3**	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52	Week 54
Visit Window (days)	-		±7	±2	±2		±2	±2	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±7
Procedure																				
Subject informed consent	X																			
IC/EC review	X		X																	
Medical history	X																			
Physical exam	X								X[A]			X[A]			X[A]				X[A]	X
Vital signs (including weight)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X		X[J]						X[J]			X[J]			X[J]				X[J]	
Fundus Photography	X																			X
Hematology[b]	X								X			X							X	X
Chemistry[c]	X								X			X							X	X
Chemistry - Lipids[d]	X	X							X			X							X	X
TSH levels	X																			
Urinalysis (includes screen for protein)	X																		X	X
UPT (WOCBP)[d]	X		X				X	X	X	X	X	X	X	X	X	X	X	X	X	
[24-hour urine protein and creatinine], if indicated by urine protein screen)	X																			X
Serology[f]	X																			
T1D antibodies[g]	X								X										X	

	Screening	Screening MMTT	*Randomization	Week 1	Week 2	Week 3**	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52	Week 54
T-cell response	X								X			X			X				X	
HbA1c	X	X							X	X		X			X				X	
4-hour MMTT/Stimulated C-peptide		X							X	X		X							X	
Fasting or nonfasting C-peptide	X																			
Continuous glucose monitoring [h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Provision of electronic diary	X																			
Study drug administration at site[i]			X	X	X	X	X													
Injection site evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Dispense study drug for at home use					X		X	X	X	X	X	X	X	X	X	X	X	X		
Collect used study drug vials							X	X	X	X	X	X	X	X	X	X	X	X	X	
Review self-monitored blood glucose (fasting)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review nighttime glucose			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review insulin use	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review hypoglycemic events	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

	Screening	Screening MMTT	*Randomization	Week 1	Week 2	Week 3**	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52	Week 54
Concomitant medication use	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Self-report of hypoglycemic events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Self-report of administration of at-home injections							X	X	X	X	X	X	X	X	X	X	X	X	x	X

[*] **Randomization could be up to 8 weeks from Screening Visit to allow for any laboratory tests to be repeated**

**** Visit for first 6 subjects in Cohort B only**

[a] **Brief Physical Exam**

[b] **Hematology:** CBC with differential and platelet count

[c] **Chemistry**: Electrolytes (sodium, potassium, chloride, bicarbonate), BUN, creatinine, glucose, albumin, calcium, AST, alkaline phosphatase, total bilirubin, total protein, creatine kinase, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides

[d] Sample for lipids should be collected in a fasted state. The screening sample will be collected at 10 minutes prior to Boost administration in the MMTT. Samples at weeks 12, 24, and 52 will be collected with other chemistry samples in 10 minutes prior to Boost administration in the MMTT. UPT results must be negative for dosing to continue

[e] Prior to study drug administration

[f] **Serology**: Hepatitis B, Hepatitis C, HIV

[g] **T1D antibodies**: GAD65, IA-2, insulin ZnT8

[h] Continuous glucose monitoring will be initiated 5 days or more before the screening MMTT visit and continued until the Week 52 visit.

[i] Study drug administration shown for in-office visits.

[J] For subjects aged 12-<18 only

APPENDIX 2: SCHEDULE OF ASSESSMENTS DURING 2-YEAR FOLLOW-UP

	Month 18	Month 24	Month 36
Visit Window (days)	±14 days	±14 days	±14 days
Procedure			
Physical exam	X	X	X
Vital signs (including weight)	X	X	X
Height (<18 years of age)	X	X	X
Fundus photograph examination		X	X
Hematology	X	X	X
Chemistry	X	X	X
HbA1c	X	X	X
Urinalysis	X	X	X
Adverse events	X	X	X
Concomitant medications	X	X	X
4-hour MMTT	X	X	X

APPENDIX 3: DECLARATION OF HELSINKI

Declaration of Helsinki (1989)

World Medical Association Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland in 1964 and as revised by the World Medical Assembly in Tokyo, Japan in 1975, in Venice, Italy in 1983, and in Hong Kong in 1989.

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that "a physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The Purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic, and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice, most diagnostic, therapeutic, or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research, a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research in which the essential object is purely scientific without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil, and ethical responsibilities under the laws of their own countries.

Basic Principles

(1) Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

(2) The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol, which should be transmitted for consideration, comment, and guidance to a specially appointed committee independent of the investigator and the Sponsor, provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

(3) Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

(4) Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

(5) Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison to foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

(6) The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

(7) Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

(8) In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

(9) In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

(10) When obtaining informed consent for the research project, the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case, the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

(11) In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes obtaining informed consent impossible, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

(12) The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

Medical Research Combined with Professional Care (Clinical Research)

(1) In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health, or alleviating suffering.

(2) The potential benefits, hazards, and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

(3) In any medical study, every patient--including those of a control group, if any--should be assured of the best proven diagnostic and therapeutic method.

(4) The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

(5) If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

(6) The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

(1) In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

(2) The subjects should be volunteers--either healthy persons or patients for whom the experimental design is not related to the patient's illness.

(3) The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

(4) In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

APPENDIX 4: STUDY CONTACT LIST

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