Protocol/Statistical Analysis Plan: I8B-MC-ITRT

Effect of Injection Site on the Relative and Absolute Bioavailability of Single Dose of LY900014 in Healthy Subjects

NCT03233983

Approval Date:

Protocol I8B-MC-ITRT(a)
Effect of Injection Site on the Relative and Absolute Bioavailability of Single Dose of LY900014 in Healthy Subjects

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LY900014

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Amendment (a) Electronically Signed and Approved by Lilly on approval date provided below.

Approval Date: 14-Jul-2017 GMT

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1. Protocol Synopsis

Title of Study:
Effect of injection site on the relative and absolute bioavailability of single dose of LY900014 in healthy subjects.

Rationale:
In clinical practice, it is recommended that patients rotate insulin injections and may inject insulin at different tissue sites. The actual site of subcutaneous (SC) injection may influence the absorption pharmacokinetics (PK), as well as, the glucodynamic (GD) effect. The primary objective of this study is therefore to compare the bioavailability of insulin lispro following administration of the commercial formulation of LY900014 at 3 different injection sites SC in the abdominal wall, thigh, and deltoid. In addition, the intravenous (IV) administration will serve as the reference to determine the absolute bioavailability of SC injections into the deltoid, thigh or abdomen and GD activity.

Objectives/Endpoints:

Objectives	Endpoints
Primary	
To determine the relative bioavailability of insulin lispro from LY900014 after SC injection into the thigh and deltoid compared to the abdomen in healthy subjects.	Area under the concentration versus time curve from time zero to infinity (AUC[0-∞])
To determine absolute bioavailability of insulin lispro following SC administrations of LY900014 into the thigh, deltoid and abdomen compared to IV administration of LY900014 in healthy subjects.	Percent of the ratio of AUC(0-∞)
Secondary	
To evaluate the tolerability of LY900014 in healthy subjects.	Adverse events (AEs) and injection-site reactions
To evaluate the GD during euglycemic clamp procedure of LY900014 after SC injection into the abdomen, thigh and deltoid and IV in healthy subjects.	Total amount of glucose infused (G_{tot}) and maximum glucose infusion rate (R_{max}).

Summary of Study Design:

Study I8B-MC-ITRT is a single-center, open-label, 4-period, randomized, crossover, up to 10-hour euglycemic clamp study in healthy subjects to compare the insulin lispro PK and GD of LY900014 after SC administration of a 15-U injection at 3 different injection sites and a single IV bolus injection of 15 U. The SC injection sites are the abdomen, thigh, and deltoid. An IV injection into the forearm will be used to assess absolute bioavailability.

Treatment Arms and Planned Duration for an Individual Subject:
Subjects will be randomly assigned to 1 of 4 treatment sequences; each sequence will have 4 periods. In 3 of the 4 periods, subjects will be administered single SC doses of 15 U of LY900014 at 3 different injection sites, which include the thigh, abdomen and deltoid. One study period will include a single IV dose of 15 U of LY900014.

The study will include a 28-day screening period, followed by 4 study periods. There will be a wash-out period of at least 3 days between each study period. The follow-up visit will take place at least 14 days after the last dose.

Number of Subjects:
Up to 28 healthy men and women may be enrolled to target at least 22 subjects complete the study.

Statistical Analysis:

The primary statistical analyses for PK will be conducted on those subjects who receive at least 1 dose of study drug and have measurable insulin lispro concentrations. The primary statistical analysis for GD will be conducted on those subjects who complete at least 1 clamp procedure. Supportive analyses will be done on the key parameters for the subjects who complete all treatment periods with evaluable data.

Safety analyses will be conducted for all enrolled subjects, whether or not they complete all protocol requirements. All investigational product and protocol-procedure AEs will be listed, and if the frequency of events allows, safety data will be summarized using descriptive methodology. Safety parameters that will be assessed include safety laboratory parameters and vital signs, as well as evaluation of injection-site reactions. The parameters will be listed and may be summarized using standard descriptive statistics.

Log-transformed AUC(0-∞) and maximum observed serum concentration (C_{max}) for insulin lispro will be evaluated to estimate geometric least-squares means (LSmeans), ratios of geometric LSmeans between injection sites (deltoid and thigh compared with the abdomen), and their corresponding 90% confidence interval (CI) using the statistical model that includes injection site, period, and sequence as fixed effects, and subject within sequence as a random effect. The primary parameter for the statistical analysis of the relative bioavailability between injection sites will be the AUC(0-∞).

Log-transformed R_{max} and G_{tot} will be evaluated to estimate geometric LSmeans, ratios of geometric LSmeans between injection sites (deltoid and thigh compared with the abdomen), and their corresponding 90% CIs using the statistical model that includes injection site, period, and sequence as fixed effects, and subject within sequence as a random effect. For GD parameters that have at least 1 subject with a value equal to zero, a value equal to the smallest non-zero observed GD value for that parameter divided by 2 will be added to all values, and the analysis of the log-transformed data will be performed. In addition, as a sensitivity analysis, a nonparametric analysis will be performed for that parameter.

The PK and GD analyses will also be performed using the subset of the subjects who complete all treatment periods with evaluable data.

The frequency of antibody formation to insulin lispro will be determined. The relationship between the presence (or absence) of antibodies and AEs will be assessed. Likewise, the relationship between the presence of antibodies and the PK parameters and GD response to insulin lispro may be assessed.

2. Schedule of Activities

Study Schedule of Protocol I8B-MC-ITRT

Procedures	Screening	Periods 1, 2, 3, 4			FU/ ED[a]	Comments
	Up to Day -28	Day -1	Day 1	Day 2		
Informed consent	X					
Admission to CRU		X				
Enrollment		X				Enrollment prior to taking first predose blood sample for the study.
Overnight fast	X	X				Subjects are expected to fast for approximately 8 hours before screening, and at least 8 hours before each dose until the end of the glucose clamp procedure.
Height	X					
Weight	X	X[#]			X	[#] Period 1 only
Hip and waist circumference		X[#]				[#] Period 1 only
Medical history and physical examination	X					
Medical assessment	X		Predose	X*	X	Medical assessment includes medical review and physical examination, as appropriate. *Prior to discharge from CRU for each period
Vital signs (supine): blood pressure and pulse rate	X		Predose, 1 hour postdose (IV period only), and 10 hours postdose (at the end of clamp procedure)		X	Predose vital signs may be collected up to 3 hours before dosing.
12-Lead ECG	X		Predose and 10 hours postdose (at the end of clamp procedure)		X	Single ECGs will be collected for safety. Predose ECGs may be collected up to 3 hours before dosing.
Clinical laboratory tests	X		Predose of Period 1. For IV period: 1 hour postdose		X	One hour postdose blood samples will be drawn only for the study period when LY900014 is administered intravenously.
Pregnancy test	X	X			X	For females of childbearing potential only. A serum pregnancy test will be performed at screening and urine pregnancy tests will be performed at all other time points.

Procedures	Screening	Periods 1, 2, 3, 4			FU/ ED[a]	Comments
	Up to Day -28	Day -1	Day 1	Day 2		
Study drug administration			0 hour			Time of study drug administration = 0 hour. Study drug will be administered at approximately the same time on Day 1 of each study period. The site of administration will be determined by the randomization schedule.
Injection-site assessments			0, 1, 4, and 10 hours postdose			Time 0: assessments will occur immediately following the injection.
Insulin lispro PK sampling			2.5, 5, 10, 15, 20, 25, 30, 40, 60, 70, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 540, and 600 minutes			Sampling times are relative to the time of study drug administration (time 0).
Treprostinil PK sampling (IV period only)			2.5, 5, 10, and 30 minutes			Only for the study period when LY900014 is administered intravenously. Sampling times are relative to the time of study drug administration (time 0).
C-peptide samples			0, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540, and 600 minutes			Sampling times are relative to the time of study drug administration (time 0).
Blood glucose sampling for euglycemic clamp			Approximately every 10 minutes for approximately 30 minutes before the start of dosing (for baseline measurement). During clamp, sampling occurs every 2.5 minutes for the first 30 minutes; every 5 minutes for 30 to 120 minutes; every 10 minutes for 120 to 480 minutes and every 20 minutes for 480 to 600 minutes.			Sampling times are relative to study drug administration (time 0). Glucose samples should be taken before PK samples. Failure to obtain samples due to clinical issues, such as problems with venous access, will not be considered a protocol violation. Repeat samples for counter-checking of apparent spurious results may be taken where indicated.
Pharmacogenetic sample		X				Period 1 only.
Immunogenicity sample			Predose of Periods 1 and 3		X	Additional samples may be collected if the investigator considers there is a possibility that an adverse event is immunologically indicated.
Discharge from CRU				X		Subjects will be discharged on Day 2.

Abbreviations: CRU = clinical research unit; ECG = electrocardiogram; ED = early discontinuation; FU = follow-up; IV = intravenous; PK = pharmacokinetics.

[a] Procedures should be performed at least 14 days after the last dose of study drug.

Note: The site should schedule activities as appropriate. In cases when several study procedures are scheduled at the same time, the order of priority will be as follows: glucose samples, PK samples, injection-site assessments, vital signs, ECG, clinical laboratory samples. If the investigator decides based on clinical judgment not to dose a subject on a given day (eg, because of low blood glucose), the subject's visit may be rescheduled; any procedures performed in that period may be repeated.

3. Introduction

3.1. Study Rationale

In clinical practice, it is recommended that patients rotate insulin injections and may inject insulin at different tissue sites. The actual site of subcutaneous (SC) injection may influence the absorption pharmacokinetics (PK), as well as, the glucodynamic (GD) effect. The primary objective of this study is therefore to compare the bioavailability of insulin lispro following administration of the commercial formulation of LY900014 at 3 different injection sites SC in the abdominal wall, thigh, and deltoid. In addition, the intravenous (IV) administration will serve as the reference to determine the absolute bioavailability of SC injections into the deltoid, thigh, or abdomen and GD activity.

3.2. Background

The insulin analog, insulin lispro (Humalog®), has been shown to be absorbed more quickly than regular human insulin (Humalog package insert, 2015). In healthy volunteers given SC doses of insulin lispro ranging from 0.1 to 0.4 U/kg, peak serum levels were seen 30 to 90 minutes after dosing (Humalog package insert, 2015). However, the general consensus is that rapid-acting insulin, administered either through pumps or syringes/pen injectors, is still not rapid enough to match carbohydrate absorption profiles, which limits efficacy and dosing flexibility. An ultra-rapid-acting prandial insulin would shift the PK and GD profiles so that they have an even faster onset to better match carbohydrate absorption.

LY900014 is an ultra-rapid-acting insulin lispro formulation with treprostinil (active ingredient in Remodulin®) and other ingredients. LY900014 has an increased early absorption of insulin lispro compared to commercially available insulin lispro (Humalog; Eli Lilly and Company). This formulation involves the novel use of a microdose of treprostinil as an excipient to enhance the absorption of insulin lispro through local vasodilatation rather than as an active pharmaceutical ingredient to elicit a systemic effect.

Treprostinil is a prostacyclin analog, administered either through inhalation (Tyvaso®), as an IV infusion, or as a continuous SC administration for the treatment of symptomatic pulmonary arterial hypertension (PAH) and has been approved in the US since 2002 (Remodulin package insert, 2014). Each of the other excipients (such as sodium citrate and magnesium chloride) in the LY900014 formulation is listed in the US Food and Drug Administration (FDA)'s Generally Recognized as Safe Food Additives database and in the FDA's Inactive Ingredients in Approved Drugs database. Furthermore, the excipient concentration in LY900014 is within the limits identified for approved drug products in the FDA Inactive Ingredients in Approved Drugs database.

Safety and tolerability of LY900014 have been demonstrated in healthy subjects in 3 previous clinical studies, in approximately 71 healthy subjects across a dose range of 7.5 to 30 U. All 4 studies were Phase 1, randomized, subject-blind studies in which the PK and GD of LY900014 and Humalog were evaluated during a euglycemic glucose clamp following SC administration. The total insulin lispro exposure and GD effect were similar for LY900014 and Humalog;

however, LY900014 demonstrated a faster and earlier insulin lispro absorption compared to Humalog.

In addition, data from two Phase 1b studies showed LY900014 was well tolerated in patients with type 1 diabetes mellitus (T1DM; 30 patients) and type 2 diabetes mellitus (T2DM; 30 patients) using multiple daily injections (MDIs). There were no serious adverse events (SAEs) related to study treatment or discontinuations from the studies because of a drug-related adverse event (AE). Small numbers of treatment-emergent adverse events (TEAEs) were reported, and there were no notable increases in these events in relation to any of the LY900014 formulations compared to those in relation to Humalog.

More information can be found in the Investigator's Brochure (IB) of LY900014.

3.3. Benefit–Risk Assessment

Study I8B-MC-ITRT (ITRT) will not offer any direct benefits to the subjects participating in the study. The data from previous studies in healthy subjects and data from studies in patients with T1DM and T2DM have shown that LY900014 was well tolerated and the adverse drug reactions are in keeping with those reported for Humalog.

Potential risks associated with LY900014, derived from the known risks of insulin lispro (Humalog), are hypoglycemia, hypersensitivity reactions (localized allergy and/or systemic allergy), undesirable effects at the injection site (injection-site reactions and lipodystrophy), and peripheral edema (Humalog package insert, 2015).

Notably, across all doses in the Lilly clinical studies that have evaluated treprostinil as a local vasodilator with or without insulin lispro, there was no clinically significant increase in those AEs associated with systemic absorption of treprostinil, as described in the Remodulin package insert (2014). The exposures of treprostinil in LY900014 in this study are expected to be undetectable compared to those observed in the dose ranges previously explored with SC bolus administration of treprostinil and are expected to be substantially lower than those observed in the treatment of PAH.

In preclinical safety pharmacology and toxicity studies, or clinical pharmacology studies involving LY900014 or treprostinil alone, other than known risks associated with Humalog and Remodulin, no additional risks were identified. No known potential risks are associated with the use of small amounts of treprostinil (Remodulin) in the LY900014 formulation.

Additionally, local and systemic toxicity profiles of Humalog and Remodulin do not suggest the potential for additive or synergistic toxicity.

Following administration of the study insulin, subjects will receive IV glucose infusion at a variable rate to maintain euglycemia up to 10 hours after insulin lispro administration. The aim of the clamp procedure is to maintain blood glucose within the normal glycemic range. In addition, the clamp is performed while the subject is inpatient and under the investigator's supervision. These considerations should minimize the risk of hypoglycemia in subjects participating in Study ITRT.

More information about the known and expected benefits, risks, SAEs, and reasonably anticipated AEs of LY900014 are to be found in the IB.

4. Objectives and Endpoints

Table ITRT.1 shows the objectives and endpoints of the study.

Table ITRT.1. Objectives and Endpoints

Objectives	Endpoints
Primary To determine the relative bioavailability of insulin lispro from LY900014 after SC injection into the thigh and deltoid compared to the abdomen in healthy subjects.	AUC(0-∞)
To determine absolute bioavailability of insulin lispro following SC administrations of LY900014 into the thigh, deltoid, and abdomen compared to IV administration of LY900014 in healthy subjects.	Percent of the ratio of AUC(0-∞)
Secondary To evaluate the tolerability of LY900014 in healthy subjects.	Adverse events and injection-site reactions
To evaluate the GD during euglycemic clamp procedure of LY900014 after SC injection into the abdomen, thigh, and deltoid and IV in healthy subjects.	G_{tot} and R_{max}
Exploratory Objectives To evaluate C-peptide levels after administration of LY900014.	C-peptide concentrations
To determine plasma concentrations of the excipient, treprostinil, following administration of a single, IV 15-U dose of LY900014 in healthy subjects.	Plasma concentrations–over-time profile

Abbreviations: AUC(0-∞) = area under the concentration versus time curve from time zero to infinity; GD = glucodynamics; G_{tot} = total amount of glucose infused; IV = intravenous; R_{max} = maximum glucose infusion rate; SC = subcutaneous.

5. Study Design

5.1. Overall Design

This is a Phase 1, single-center, open-label, 4-period, randomized, crossover, up to 10-hour euglycemic clamp study in approximately 28 healthy subjects to compare the insulin lispro PK and GD of LY900014 after SC administration of a 15-U dose at 3 different injection sites and a single IV bolus injection of 15 U. The SC injection sites are the abdomen, thigh, and deltoid.

Subjects will be required to attend the clinical research unit (CRU) on at least 6 occasions:

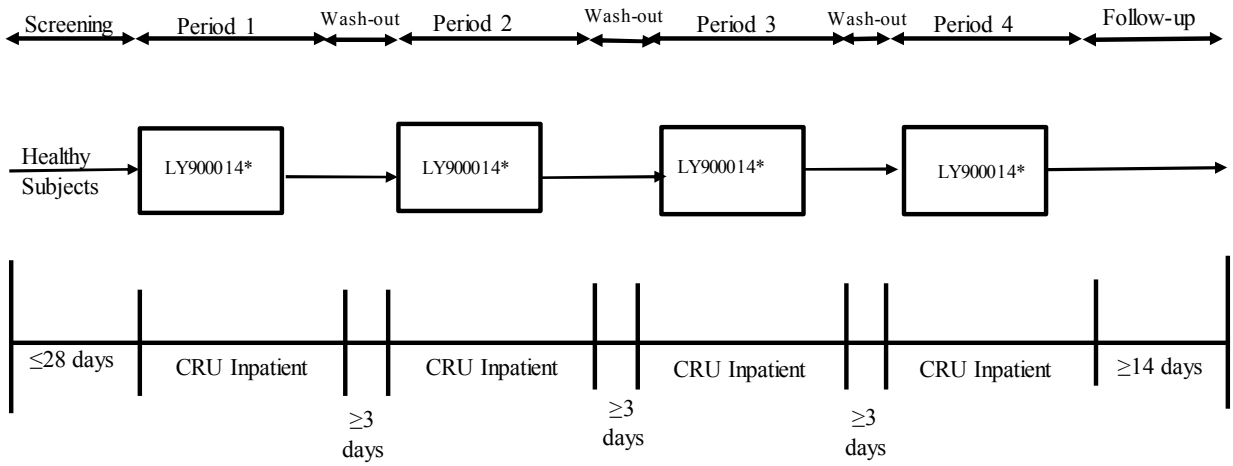
- screening visit (may occur up to 28 days before randomization)
- four treatment visits for the clamp procedure (study Periods 1 to 4) with a wash-out period of ≥3 days between discharge and the next admission to the CRU
- follow-up visit (at least 14 days after last dose), or early discontinuation.

Each subject will be randomly assigned to a treatment sequence and will be administered single doses of 15 U of LY900014 (on 4 occasions).

Subjects will be admitted to the CRU on the evening before each dosing day and will remain in the CRU for the duration of the clamp period and until discharge by the investigator. Subjects are expected to fast for at least 8 hours before each dose. Following dose administration, each subject will undergo an euglycemic clamp procedure of up to 10 hours. Upon completion of the clamp procedures, the subjects will be provided a meal and observed overnight. Subjects will be discharged from the CRU the next day after medical assessments.

Study governance considerations are described in detail in Appendix 3.

Figure ITRT.1 illustrates the study design and Table ITRT.2 presents the treatment sequences.



Abbreviations: CRU = clinical research unit; IV = intravenous; SC = subcutaneous.
*Single dose of LY900014 administered either SC in the abdomen, thigh, deltoid or IV. Up to 10-hour euglycemic clamp procedure after each dose.

Figure ITRT.1. Illustration of study design of Protocol I8B-MC-ITRT.

Table ITRT.2. LY900014 Treatment Sequences

Treatment Sequence	Period 1	Period 2	Period 3	Period 4
1	LY900014 Abdomen	LY900014 Thigh	LY900014 Deltoid	LY900014 IV
2	LY900014 Deltoid	LY900014 Abdomen	LY900014 IV	LY900014 Thigh
3	LY900014 IV	LY900014 Deltoid	LY900014 Thigh	LY900014 Abdomen
4	LY900014 Thigh	LY900014 IV	LY900014 Abdomen	LY900014 Deltoid

Abbreviation: IV = intravenous.
Note: Subcutaneous injection sites are the abdomen, deltoid and thigh.

5.2. Number of Participants

A total of 28 subjects may be enrolled so that at least 22 subjects complete the study. For purposes of this study, a subject completes the study when all scheduled procedures shown in the Schedule of Activities (Section 2) have been completed.

If subjects discontinue from the study before completion of all 4 study periods, replacement subjects may be enrolled up to 28 subjects following agreement between the investigator and the sponsor.

A replacement subject will be assigned the treatment sequence of the discontinued subject and complete that treatment sequence in its entirety.

5.3. End of Study Definition

End of the study is the date of the last visit or last scheduled procedure shown in the Schedule of Activities (Section 2) for the last subject.

5.4. Scientific Rationale for Study Design

A population of healthy subjects is selected based on the likelihood of less physiologic variability in the absence of disease states that may affect multiple organ systems and absence of other confounding factors such as concomitant medications.

The use of a crossover design allows each subject to serve as his or her own control, thereby reducing variability.

A euglycemic clamp technique, the gold standard methodology for assessing insulin action, will be used in this study to provide data on the GD activity of LY900014 (see Section 9.6 for a detailed description of the clamp methodology). Based on previous studies, the 10-hour duration of the euglycemic glucose clamp will allow for a complete assessment of GD activity of LY900014.

Based on the PK properties of treprostinil (half-life associated with the terminal rate constant in noncompartmental analysis [$t_{1/2}$] = approximately 1 hour) and Humalog ($t_{1/2}$ = 0.79 hours), the duration of the washout period between clamp visits (a minimum of 3 days) and the duration between the last dose of study drug and the follow-up visit (at least 14 days) are considered appropriate.

5.5. Justification for Dose

Based on previous studies of both insulin lispro (Humalog) and LY900014, the 15-U dose is within the clinical dose range and should provide measurable PK and GD profiles for insulin lispro. The insulin lispro concentrations in the body resulting from administration of this dose to healthy subjects are anticipated to be measurable over the sampling period. Additionally, it is anticipated that this dose will provide an adequate glucose infusion rate (GIR) for assessment of GD.

The safety, PK, and pharmacology of LY900014 at similar doses and with the similar formulation composition have been assessed in clinical studies in healthy subjects, in patients with T1DM using MDI or insulin pump treatment and T2DM using MDI. In addition, the components of LY900014 have been tested in 3 clinical studies that included the evaluation of the safety, PK, and pharmacology of SC bolus doses of treprostinil (see LY900014 IB).

All tested doses of treprostinil (CCI [redacted]), insulin lispro, and LY900014 were well tolerated in healthy subjects, patients with T1DM, and patients with T2DM. There were no SAEs related to study treatment in any of the 8 studies. No subject discontinued from the studies because of drug-related AEs. Trial participants in these studies were monitored for changes in vital signs; there were no significant systemic hemodynamic effects of treprostinil at the doses administered based on blood pressure and heart rate. Visual analog scale pain scores showed that the SC injections of insulin lispro plus treprostinil co-formulations and treprostinil alone were well tolerated.

In summary, there were small numbers of TEAEs and injection-site AEs, but there was no clinically significant increase in these or other events compared to placebo or to Humalog and no

clinically significant increase in frequency with higher doses of treprostinil. Notably, at the higher doses of treprostinil, there was no clinically significant increase in those AEs associated with systemic absorption as described in the Remodulin package insert (2014) (that is, headache, diarrhea, nausea, jaw pain, vasodilatation, rash, edema, and hypotension). This is likely attributable to the relatively lower exposures of treprostinil demonstrated in these clinical studies.

It is expected that the treprostinil concentration in the plasma following the 15-U IV bolus administration of LY900014 containing CCI of treprostinil to be below the lower limit of quantitation (LLOQ). As published, the SC infusion of Remodulin has a 100% absolute bioavailability (Remodulin package insert, 2014); thus, it is expected that plasma exposures of treprostinil between IV and SC administrations of LY900014 will be similar. The PK of treprostinil in LY900014 following SC administration of 15 U of LY900014 was assessed in previous studies. In these studies, treprostinil exposure was not detectable for the 15-U dose of LY900014.

6. Study Population

Eligibility of subjects for the study will be based on the results of screening medical history, physical examination, vital signs, clinical laboratory tests, and electrocardiogram (ECG).

The nature of any conditions present at the time of the physical examination and any preexisting conditions will be documented.

Screening may occur up to 28 days prior to enrollment. Subjects who are not enrolled within 28 days of screening may be subjected to an additional medical assessment and/or clinical measurements to confirm their eligibility.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Inclusion Criteria

Subjects are eligible for inclusion in the study only if they meet all of the following criteria at screening:

[1] are overtly healthy males or females, as determined by medical history and physical examination

[1a] female subjects:

For female subjects of childbearing potential (defined as not surgically sterilized and between menarche and 1-year postmenopause):

- test negative for pregnancy at the time of screening
- are not lactating
- intend not to become pregnant during the study
- are sexually inactive or have practiced a reliable method of birth control (eg, use of oral contraceptives or levonorgestrel, diaphragms with contraceptive jelly, cervical caps with contraceptive jelly, condoms with contraceptive foam, intrauterine devices, partner with vasectomy, or abstinence) for at least 6 weeks prior to screening
- agree to continue to use a reliable method of birth control (as determined by the investigator) until the end of the study

Female subjects not of childbearing potential due to surgical sterilization (at least 6 weeks after surgical bilateral oophorectomy with or without hysterectomy or at least 6 weeks after tubal ligation) confirmed by medical history or due to menopause.

- Menopausal women include women with either

1) spontaneous amenorrhea for at least 12 months, not induced by a medical condition such as anorexia nervosa and not taking medications during the amenorrhea that induced the amenorrhea (eg, oral contraceptives, hormones,

gonadotropin-releasing hormone, antiestrogens, selective estrogen receptor modulators, or chemotherapy)

or

2) spontaneous amenorrhea for 6 to 12 months and a follicle-stimulating hormone level consistent with menopausal state

[2] are between 21 and 65 years of age at the time of screening, inclusive.

[3] have a body weight of ≥45 kg, and body mass index (BMI) of 18.0 to 30.0 kg/m^2, inclusive

[4] have clinical laboratory test results within normal reference range for the population or CRU, or results with acceptable deviations that are judged to be not clinically significant by the investigator.

[5] have venous access sufficient to allow for blood sampling, IV administration, and clamp procedure as per the protocol.

[6] are nonsmokers, have not smoked for at least 6 months before entering the study, and agree not to smoke (cigars, cigarettes, or pipes) or use smokeless tobacco or nicotine products for the duration of the study.

[7] are reliable and willing to make themselves available for the duration of the study and are willing to follow study procedures.

[8] are able and willing to give signed informed consent.

6.2. Exclusion Criteria

Subjects will be excluded from study enrollment if they meet any of the following criteria at screening and/or enrollment:

[9] are CRU personnel directly affiliated with this study and their immediate families. Immediate family is defined as a spouse, biological or legal guardian, child, or sibling.

[10] are Lilly employees.

[11] are currently enrolled in a clinical study involving an investigational product (IP) or any other type of medical research judged not to be scientifically or medically compatible with this study.

[12] have participated, within the last 30 days, in a clinical study involving an IP. If the previous IP has a long half-life, 5 half-lives, or 3 months (whichever is longer) should have passed.

[13] have previously completed or withdrawn from this study.

[14] have known allergies to treprostinil or insulin lispro, related compounds, or any components of the formulation, or history of significant atopy.

[15] have an abnormality in the 12-lead ECG that, in the opinion of the investigator, increases the risks associated with participating in the study.

[16] have an abnormal blood pressure and/or pulse rate as deemed to be clinically significant by the investigator.

[17] have a history or presence of cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; of constituting a risk when taking the IP; or of interfering with the interpretation of data.

[18] have a history or presence of psychiatric disorders as deemed clinically significant by the investigator.

[19] regularly use known drugs of abuse.

[20] show evidence of human immunodeficiency virus (HIV) infection, and/or positive human HIV antibodies.

[21] show evidence of hepatitis B and/or positive hepatitis B surface antigen.

[22] show evidence of hepatitis C and/or positive hepatitis C antibody.

[23] use over-the-counter or prescription medication within 7 to 14 days, respectively, prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, thyroid replacement medication, or birth control methods) and throughout the study period. If this situation arises, inclusion of an otherwise suitable subject may be at the discretion of the investigator and sponsor.

[24] have donated blood or have blood loss of more than 450 mL within the previous 3 months of study screening.

[25] have an average weekly alcohol intake that exceeds 21 units per week (males) and 14 units per week (females), or are unwilling to stop alcohol consumption from 24 hours prior to dosing and until discharge from the CRU (1 unit = 12 oz or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45 mL of distilled spirits).

[26] in the opinion of the investigator or sponsor, are unsuitable for inclusion in the study.

Additional inclusion criterion:

[27] have a hemoglobin level of ≥12.5 g/dL at screening.

6.3. Lifestyle and/or Dietary Requirements

Throughout the study, subjects may undergo medical assessments and review of compliance with requirements before continuing in the study.

6.3.1. Meals and Dietary Restrictions

For all treatment periods subjects will fast for at least 8 hours prior to dosing and until the glucose clamp procedure is completed, after which subjects will receive a meal. Water can be consumed freely during this time.

When not a resident at the CRU, subjects will be encouraged to follow their normal diets.

6.3.2. Caffeine, Alcohol, and Tobacco

No alcohol will be allowed at least 24 hours before each dose and for the duration of each CRU visit.

Subjects should refrain from caffeine-containing food/beverages (eg, cola, chocolate, Milo, tea, and coffee) for at least 12 hours before each dose and throughout the duration of each CRU visit.

Smoking (cigars, cigarettes, or pipes) and the use of smokeless tobacco will not be permitted during the study.

6.3.3. Activity

Subjects are encouraged to maintain their regular exercise habits; however, they should not undertake vigorous or prolonged exercise within 48 hours prior to dosing. These subjects will be excluded from this study, as judged by the investigator to prevent interference with study results. After dosing, subjects should remain recumbent or sitting in the CRU until the end of the glucose clamp.

Movement will be restricted to retain the integrity of connections to the infusion(s) and the study procedures.

6.4. Screen Failures

Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened.

7. Treatment

7.1. Treatment Administered

The study involves a comparison of LY900014 administered once through SC at different injection sites which include, the abdomen, thigh, and deltoid and administered once through IV in the forearm. Table ITRT.3 shows the treatment regimens.

The CRU must have resuscitation equipment, emergency drugs, and appropriately trained staff available during the IP administration.

Table ITRT.3. Treatments Administered

Treatment Name	LY900014
Insulin lispro dosage formulation	100 U/mL
Insulin lispro dosage level	15 U
Treprostinil concentration (treprostinil dose)	CCI [redacted]
Route of administration	SC injection or IV bolus

Abbreviations: IV = intravenous; SC = subcutaneous.

The investigator or designee is responsible for

- explaining the correct use of the IP to the CRU personnel
- verifying that instructions are followed properly
- maintaining accurate records of IP dispensation and collection
- and returning all unused medications to Lilly or its designee at the end of the study

Note: In some cases, the CRU may destroy the material if, during the investigative site selection, the evaluator has verified and documented that the CRU has appropriate facilities and written procedures to dispose of clinical materials.

7.1.1. Packaging and Labeling

Clinical study materials will be labeled according to the country's regulatory requirements. LY900014 will be supplied by Lilly or its representative in accordance with current good manufacturing practices and will be supplied with lot numbers.

Each vial will contain CCI [redacted]. A pharmacist at the CRU or other CRU personnel will use the insulin vials provided to prepare the syringes for dose administrations.

7.2. Method of Treatment Assignment

The injection site where LY900014 is to be injected on a given treatment day will be determined according to a randomization schedule.

7.2.1. Selection and Timing of Doses

The actual date and time of all dose preparations will be documented, and the actual time of all dose administrations will be recorded in the subject's electronic case report form (eCRF). For each subject, the doses will be administered at approximately the same time on Day 1 of each study period.

Subjects will receive LY900014 at the injection site specified in the treatment sequence to which they have been randomized. The SC injection site shall be either a lower quadrant of the abdominal wall, nondominant upper lateral arm, or the anterior midline of the thigh or subjects will receive one IV dose of LY900014. An appropriate size of needle shall be used to ensure all injections are delivered to a consistent depth target into the SC space.

The IV administration of LY900014 will be a bolus push through a cannula into a vein in the forearm, followed immediately by flushing with normal saline.

All study treatments will be given in the CRU by qualified CRU personnel as designated by the investigator. Study injections should be given by a limited number of individuals for consistency.

The site of administration of each injection will be recorded.

7.3. Blinding

This is an open-label study.

7.4. Dose Modification

Dose adjustments are not allowed in this study.

7.5. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained, as communicated by the sponsor, during transit for all IPs received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive IP, and only authorized CRU staff may supply or administer IP. All IPs should be stored in an environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized CRU staff.

The IP must be stored at the CRU under refrigerated conditions (between 2°C to 8°C) in a locked and secure place. Insulin must not be frozen.

The investigator is responsible for the IP accountability, reconciliation, and record maintenance (such as receipt, reconciliation, and final disposition records).

7.6. Treatment Compliance

The IP will be administered at the CRU, and documentation of treatment administration will occur at the CRU.

7.7. Concomitant Therapy

Subjects should not use over-the-counter or prescription medications within 7 and 14 days, respectively, before each dosing (apart from vitamin and mineral supplements, occasional paracetamol, thyroid replacement therapy, or birth control medication) and throughout the study period. If a subject does use these medications, inclusion of the subject may be at the discretion of the investigator and sponsor.

If the need for concomitant medication arises, inclusion or continuation of the subject may be at the discretion of the investigator, and when possible, after consultation with a Lilly clinical pharmacologist (CP)/clinical research physician (CRP). Any additional medication used during the course of the study must be documented.

7.8. Treatment after the End of the Study

Not applicable.

8. Discontinuation Criteria

Subjects discontinuing the IP and/or study prematurely for any reason should complete procedures per Section 2 of this protocol.

8.1. Discontinuation from Study Treatment

8.1.1. Discontinuation of Inadvertently Enrolled Subjects

If the sponsor or investigator identifies a subject who did not meet enrollment criteria and was inadvertently enrolled, a discussion must occur between the Lilly CP/CRP and the investigator to determine if the subject may continue in the study. If both agree it is medically appropriate to continue, the investigator must obtain documented approval from the Lilly CP/CRP to allow the inadvertently enrolled subject to continue in the study with or without continued treatment with IP.

8.2. Discontinuation from the Study

Subjects will be discontinued under the following circumstances:

- Enrollment in any other clinical study involving an IP or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study
- Participation in the study needs to be stopped for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP)
- Investigator Decision
    - the investigator decides that the subject should be discontinued from the study
- Subject Decision
    - the subject, or legal representative, requests to be withdrawn from the study.

8.3. Subjects Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the CRU. The CRU personnel are expected to make diligent attempts to contact subjects who fail to return for a scheduled visit or were otherwise unable to be followed up by the CRU.

9. Study Assessments and Procedures

Section 2 lists the Schedule of Activities, detailing the study procedures and their timing (including tolerance limits for timing).

Appendix 2 lists the laboratory tests that will be performed for this study.

Appendix 4 provides a summary of the maximum number and volume of invasive samples, for all sampling, during the study.

Unless otherwise stated in the subsections below, all samples collected for specified laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

9.1. Efficacy Assessments

Not applicable for this study.

9.2. Adverse Events

Investigators are responsible for monitoring the safety of subjects who have entered this study and for alerting Lilly or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the subject.

The investigator is responsible for the appropriate medical care of subjects during the study.

Investigators must document their review of each laboratory safety report.

The investigator remains responsible for the following, through an appropriate health care option, AEs that are serious or otherwise medically important, considered related to the IP or the study, or that caused the subject to discontinue the IP before completing the study. The subject should be followed until the event resolves, stabilizes with appropriate diagnostic evaluation, or is reasonably explained. The frequency of follow-up evaluations of the AE is left to the discretion of the investigator.

After the informed consent form (ICF) is signed, CRU personnel will record, via electronic data entry, the occurrence and nature of each subject's preexisting conditions, including clinically significant signs and symptoms of the disease under treatment in the study. Additionally, CRU personnel will record any change in the condition(s) and the occurrence and nature of any AEs.

The investigator will interpret and document whether or not an AE has a reasonable possibility of being related to study treatment, or a study procedure, taking into account the disease, concomitant treatment, or pathologies.

A "reasonable possibility" means that there is a potential cause and effect relationship between the IP, and/or study procedure and the AE.

Planned surgeries should not be reported as AEs unless the underlying medical condition has worsened during the course of the study.

If a subject's IP is discontinued as a result of an AE, CRU personnel must report this to Lilly or its designee via electronic data entry.

9.2.1. Serious Adverse Events

An SAE is any AE from this study that results in 1 of the following:

- death
- initial or prolonged inpatient hospitalization
- a life-threatening experience (that is, immediate risk of dying)
- persistent or significant disability/incapacity
- congenital anomaly/birth defect
- important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent 1 of the other outcomes listed in the definition above.

The CRU personnel must alert the Lilly CRP/CP, or its designee, of any SAE as soon as practically possible.

Additionally, CRU personnel must alert Lilly Global Patient Safety, or its designee, of any SAE within 24 hours of investigator awareness of the event via a sponsor-approved method. If alerts are issued via telephone, they are to be immediately followed with official notification on study-specific SAE forms. This 24-hour notification requirement refers to the initial SAE information and all follow-up SAE information.

Although all AEs are recorded in the electronic data entry after signing informed consent, SAE reporting to the sponsor begins after the subject has signed informed consent and has received IP. However, if an SAE occurs after signing ICF, but prior to receiving IP, AND is considered Reasonably Possibly Related to a study procedure then it MUST be reported.

Investigators are not obligated to actively seek AEs or SAEs in subjects once they have discontinued from and/or completed the study (the subject summary eCRF has been completed). However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably possibly related to the study treatment or study participation, the investigator must promptly notify Lilly.

Pregnancy (maternal or paternal exposure to IP) does not meet the definition of an AE. However, to fulfill regulatory requirements any pregnancy should be reported following the SAE process to collect data on the outcome for both mother and fetus.

9.2.1.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the IB and that the investigator reports as related to IP or procedure. Lilly has procedures that

will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

9.2.2. Complaint Handling

Lilly collects product complaints on IPs and drug delivery systems used in clinical studies in order to ensure the safety of study participants, monitor quality, and to facilitate process and product improvements.

Subjects should be instructed to contact the investigator as soon as possible if he or she has a complaint or problem with the IP so that the situation can be assessed.

9.3. Treatment of Overdose

For the purposes of this study, an overdose of LY900014 is considered any dose higher than the dose assigned through randomization.

Excess insulin administration may cause hypoglycemia and hypokalemia. Mild episodes of hypoglycemia usually can be treated with oral glucose. More severe episodes with coma, seizure, or neurologic impairment may be treated with intramuscular/SC glucagon or concentrated IV glucose. Sustained carbohydrate intake and observation may be necessary because hypoglycemia may recur after apparent clinical recovery. Hypokalemia must be corrected appropriately.

9.4. Safety

9.4.1. Laboratory Tests

For each subject, laboratory tests detailed in Appendix 2 should be conducted according to the Schedule of Activities (Section 2).

9.4.2. Vital Signs

For each subject, vital sign measurements should be conducted according to the Schedule of Activities (Section 2) and as clinically indicated.

Blood pressure and pulse rate should be measured after at least 5 minutes supine.

Unscheduled orthostatic vital signs should be assessed, if possible, during any AE of dizziness or posture-induced symptoms. Additional vital signs may be measured during each study period if warranted.

9.4.3. Electrocardiograms

For each subject, ECGs should be collected according to the Schedule of Activities (Section 2).

Any clinically significant findings from ECGs that result in a diagnosis and that occur after the subject receives the first dose of the IP, should be reported to Lilly, or its designee, as an AE via electronic data entry methods.

For each subject, a single 12-lead digital ECG will be collected according to the Schedule of Activities (Section 2). Subjects must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake during ECG collection. Electrocardiograms may be obtained at additional times, when deemed clinically necessary. All ECGs recorded should be stored at the CRU.

Electrocardiograms will be interpreted by a qualified physician (the investigator or qualified designee) at the CRU as soon after the time of ECG collection as possible, and ideally while the subject is still present, to determine whether the subject meets entry criteria at the relevant visit(s) and for immediate subject management, should any clinically relevant findings be identified.

If a clinically significant finding is identified (including, but not limited to, changes in QT/corrected QT interval from baseline) after enrollment, the investigator will determine if the subject can continue in the study. The investigator, or qualified designee, is responsible for determining if any change in subject management is needed, and must document his/her review of the ECG printed at the time of collection. Any new clinically relevant finding should be reported as an AE.

9.4.4. Other Tests

9.4.4.1. Physical Examinations

Physical examinations and routine medical assessments will be conducted as specified in the Schedule of Activities (Section 2) and as clinically indicated.

9.4.4.2. Body Weight

Body weight will be recorded as specified in the Schedule of Activities (Section 2) and as clinically indicated.

9.4.4.3. Hip and Waist Circumference

Hip and waist circumference will be recorded as specified in the Schedule of Activities (Section 2). The average of triplicate measurements of waist (narrowest circumference between lowest aspect of the ribs and anterior superior iliac crests) and the hip (widest circumference between the anterior superior iliac crests and the greater trochanters) circumference will be measured.

9.4.5. Safety Monitoring

The Lilly CP or CRP/scientist will monitor safety data throughout the course of the study.

Lilly will review SAEs within time frames mandated by company procedures. The Lilly CP or CRP will periodically review all safety data including laboratory analytes and AEs.

When appropriate, the Lilly CP or CRP will consult with the functionally independent Global Patient Safety therapeutic area physician or clinical research scientist.

9.4.5.1. Glucose Monitoring

Hypoglycemia will be described using the following definitions:

- **Documented hypoglycemia:**
  - **Symptomatic hypoglycemia:** an event during which typical symptoms of hypoglycemia are accompanied by plasma glucose ≤70 mg/dL (3.9 mmol/L)
  - **Asymptomatic hypoglycemia:** an event not accompanied by typical symptoms of hypoglycemia but with plasma glucose ≤70 mg/dL (3.9 mmol/L)
- **Unspecified hypoglycemia:** an event during which plasma glucose ≤70 mg/dL (3.9 mmol/L) but no information relative to symptoms of hypoglycemia was recorded
- **Probable symptomatic hypoglycemia:** an event during which symptoms indicative of hypoglycemia are not accompanied by a plasma glucose determination (but that was presumably caused by plasma glucose ≤70 mg/dL [3.9 mmol/L])
- **Severe hypoglycemia:** an event requiring assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. During these episodes, the subject has an altered mental status and cannot assist in their care, is semiconscious or unconscious, or experienced coma with or without seizures and may require parenteral therapy. Plasma glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of blood glucose concentration to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration (≤70 mg/dL [3.9 mmol/L])
- **Nocturnal hypoglycemia:** any hypoglycemic event (documented symptomatic, asymptomatic, probable symptomatic, or severe hypoglycemia) that occurs between bedtime and waking
- **Relative hypoglycemia:** an event during which typical symptoms of hypoglycemia, that do not require the assistance of another person, are accompanied by plasma glucose >70 mg/dL (3.9 mmol/L), but these levels may be quickly approaching the 70-mg/dL (3.9-mmol/L) threshold
- **Total hypoglycemia:** This optional category combines all cases of hypoglycemia. If an event of hypoglycemia falls into multiple subcategories, the event is counted only once in this category

The goal of the euglycemic clamp is to maintain glucose concentrations at normoglycemic levels close to a predefined target. Therefore, plasma glucose concentrations below 70 mg/dL will not routinely be recorded as hypoglycemic events during the glucose clamp procedure. However, at the discretion of the investigator, decrease in glucose concentrations may be recorded as a hypoglycemic event based on clinical concern or related to technical issues resulting in hypoglycemia.

9.4.5.1.1. Severe Hypoglycemia

The determination of a hypoglycemic event as an episode of severe hypoglycemia as defined above will be made by the investigator based on the medical need of the subject to have required assistance and is not predicated on the report of a subject simply having received assistance.

Only severe hypoglycemia episodes will be reported as AEs. All episodes of severe hypoglycemia will be reported as SAEs.

9.4.6. Injection-Site Assessments (Local Tolerability)

Injection-site assessments for local tolerability will be conducted as specified in the Schedule of Activities (Section 2) and more frequently if deemed necessary by the investigator.

Digital pictures will be taken, if possible, of the injection site at the time of identification of local intolerability and thereafter as often as judged necessary by the investigator. The pictures should include subject number, visit number, time after dosing, and a ruler for scaling.

Local tolerability at the injection site will be evaluated by means of assessments within the following categories: pain on palpation, itching, erythema, edema, and induration/infiltration.

9.5. Pharmacokinetics

At the visits and times specified in the Schedule of Activities (Section 2), venous blood samples of approximately 1 mL each will be collected to determine the serum concentrations of insulin lispro and samples of approximately 2 mL each will be collected to determine plasma concentrations of treprostinil.

Instructions for the collection and handling of blood samples will be provided by the sponsor. The actual date and time (24-hour clock) of each sampling will be recorded. Failure to obtain blood samples due to clinical reasons, such as problems with venous access, will not be considered protocol violations. However, the CRU will still be required to notify the sponsor in writing to account for missing samples for data reconciliation purpose.

9.5.1. Bioanalysis

Samples will be analyzed at a laboratory approved by the sponsor and stored at a facility designated by the sponsor.

Concentrations of insulin lispro in serum following LY900014 administration will be assayed using a CCI [redacted]. Concentrations of treprostinil in plasma following LY900014 IV administration will be assayed using a validated CCI [redacted] CCI [redacted].

Bioanalytical samples collected to measure insulin lispro and treprostinil concentrations will be retained for a maximum of 1 year following the last subject visit for the study.

9.6. Glucodynamics (Euglycemic Glucose Clamp)

The aim of the euglycemic glucose clamp is to maintain target glucose levels through infusion of a 20% d-glucose (dextrose) solution after the administration of a dose of insulin. During the glucose clamp, the GIR will be adjusted to maintain a predetermined target blood glucose concentration for the individual subject. Thus, blood glucose concentrations are kept constant while the GIR varies. The varying GIR will then reflect the GD activity of insulin.

All glucose clamp procedures will be performed after an overnight fast of at least 8 hours. On the morning of each study period, a small catheter will be placed into a forearm vein, ideally at the elbow, for infusion of glucose. Another catheter will be placed at the wrist or hand or, in the case of difficult venous access, in the forearm as close to the wrist as possible for blood sampling. This area will be heated with a warming device to approximately 55°C to 60°C for sampling arterialized venous blood. Blood samples will be obtained at the bedside for immediate determination of whole blood glucose concentrations using an automated glucose oxidase technique or other appropriate analytical method. These glucose measurements shall be used for subject safety management as well as for GD evaluations.

The time of administration of LY900014 will be defined as time zero. Following completion of dosing, in conjunction with frequent blood sampling for measurement of blood glucose, 20% dextrose will be infused IV at a variable rate in order to maintain euglycemia up to 10 hours after LY900014 administration.

The clamp procedure will continue for up to 10 hours after dose or until after blood glucose concentrations return to baseline without any glucose being administered for at least 30 minutes, whichever is earlier.

Sampling for blood glucose should occur as described in the Schedule of Activities (Section 2). Repeat samples for counter-checking of apparent spurious results may be taken where indicated. Three or 4 predose glucose values will be used for calculation of mean predose fasting blood glucose (FBG) concentration.

The target value for blood glucose concentrations is defined as 5 mg/dL below the mean of predose FBG concentration measured on the day of the glucose clamp. Subjects will not be clamped to a glucose target of lower than 63 mg/dL (whole blood). Therefore, subjects with a mean predose FBG less than 68 mg/dL (equivalent to a fasting plasma glucose of 76 mg/dL [4.21 mmol/L]) will not undergo the clamp procedure but may be deferred to a later period. In addition, any study procedures conducted up to that time may be repeated in that later period.

The GIRs required to maintain target glucose levels and blood glucose concentrations will be documented throughout the procedure. Subjects will be medically assessed before discharge from the CRU (see Schedule of Activities [Section 2]).

9.6.1. Immunogenicity Assessments

Blood samples for immunogenicity testing will be collected to determine antibody production against insulin lispro as described in the Schedule of Activities (Section 2). Additional samples may be collected if there is a possibility that an AE is immunologically mediated.

Immunogenicity will be assessed by a validated assay designed to detect antidrug antibodies in the presence of insulin lispro.

Samples will be retained for a maximum of 15 years after the last subject visit, or for a shorter period if local regulations and ethical review boards (ERBs) allow, at a facility selected by the

sponsor. The duration allows the sponsor to respond to future regulatory requests related to the IP. Any samples remaining after 15 years will be destroyed.

9.7. Genetics

A blood sample will be collected for pharmacogenetic analysis as specified in the Schedule of Activities (Section 2), where local regulations allow.

Samples will not be used to conduct unspecified disease or population genetic research either now or in the future. Samples will be used to investigate variable exposure or response to the study drug and to investigate genetic variants thought to play a role in diabetes mellitus. Assessment of variable response may include evaluation of AEs.

All samples will be coded with the subject number. These samples and any data generated can be linked back to the subject only by the CRU personnel.

Samples will be retained for a maximum of 15 years after the last subject visit, or for a shorter period if local regulations and/or ERBs impose shorter time limits, for the study at a facility selected by Lilly. This retention period enables use of new technologies, response to regulatory questions, and investigation of variable response that may not be observed until later in the development of LY900014 or after LY900014 is commercially available.

Molecular technologies are expected to improve during the 15-year storage period and therefore cannot be specifically named. However, existing approaches include whole genome or exome sequencing, genome-wide association studies, multiplex assays, and candidate gene studies. Regardless of technology utilized, data generated will be used only for the specific research scope described in this section.

9.8. Exploratory Biomarkers

Blood samples will be obtained for determination of C-peptide concentrations as specified in the Schedule of Activities (Section 2). These samples and any remaining serum after C-peptide analyses will be discarded. Instructions for the collection and handling of these samples will be provided by the sponsor.

9.9. Health Economics

This section is not applicable for this study.

10. Statistical Considerations and Data Analysis

10.1. Sample Size Determination

Up to 28 subjects may be enrolled to ensure that at least 22 subjects complete the study. Twenty-two completing subjects will provide estimated 2-sided 90% confidence intervals (CIs) of the ratios of geometric means for AUC[0-∞] after SC injection into the thigh and deltoid, compared to the abdomen, to be within approximately 0.8 to 1.25 when the observed ratio is 1. This calculation is based on the assumption of a log-normal distribution and an estimate of intrasubject log-scale standard deviation of 0.2.

10.2. Populations for Analyses

10.2.1. Study Participant Disposition

A detailed description of subject disposition will be provided at the end of the study.

10.2.2. Study Participant Characteristics

Standard baseline characteristics of age, sex, ethnicity, race, height, weight, and BMI will be summarized for all randomized subjects.

10.3. Statistical Analyses

Statistical analysis of this study will be the responsibility of Eli Lilly and Company or its designee.

The primary statistical analyses for PK will be conducted on those subjects who receive at least 1 dose of study drug and have measurable insulin lispro concentrations. The primary statistical analysis for GD will be conducted on those subjects who complete at least 1 clamp procedure. Supportive analyses will be done on the key parameters for the subjects who complete all treatment periods with evaluable data.

10.3.1. Safety Analyses

Safety analyses will be conducted for all enrolled subjects, whether or not they completed all protocol requirements.

Any change to the data analysis methods described in the protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol and the justification for making the change will be described in the study results.

Additional exploratory analyses of the data will be conducted as deemed appropriate. Study results may be pooled with the results of other studies for population PK analysis purposes to avoid issues with post hoc analyses and incomplete disclosures of analyses.

10.3.1.1. Clinical Evaluation of Safety

All IP and protocol procedure AEs will be listed, and if the frequency of events allows, safety data will be summarized using descriptive methodology.

The incidence of symptoms for each treatment will be presented by severity and by association with IP as perceived by the investigator. Symptoms reported to occur prior to enrollment will be distinguished from those reported as new or increased in severity during the study. Each symptom will be classified by the most suitable term from the Medical Dictionary for Regulatory Activities.

The number of IP-related SAEs will be reported.

10.3.1.2. Statistical Evaluation of Safety

Safety parameters that will be assessed include safety laboratory parameters and vital signs, as well as evaluation of injection-site reactions. The parameters will be listed and may be summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

10.3.2. Pharmacokinetic Analyses

10.3.2.1. Pharmacokinetic Parameter Estimation

Insulin lispro PK parameter estimates for LY900014 will be calculated using standard noncompartmental methods of analysis.

Free serum insulin lispro concentrations will be used to calculate several PK parameters, including maximum observed drug concentration (C_{max}), time of maximum observed drug concentration (t_{max}), $t_{1/2}$, area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration (AUC[0-tlast]), AUC from time zero to 10 hours (AUC[0-10h]), area under the concentration versus time curve from time zero to infinity (AUC[0-∞]), apparent total body clearance of drug calculated after extra-vascular administration (CL/F), and apparent volume of distribution during the terminal phase after extra-vascular administration (Vz/F) will be determined. In addition, the total body clearance of drug calculated (CL), and volume of distribution (V) after IV administration also will be determined. Other parameters may be calculated as deemed appropriate. Additional partial AUCs may be computed as necessary.

Additional model-based analysis will be conducted as deemed appropriate to describe the elimination and absorption profiles.

Given the predicted low systemic concentrations of treprostinil, the PK analysis of treprostinil from the IV LY900014 dosing will be conducted using the available data. The primary analysis of the treprostinil concentrations will be focused on assessing t_{max} and C_{max}.

Although attempts will be made to adhere to the scheduled collection times (Section 2), it is recognized that situations arise that may compromise the scheduled times. Parameters will be individually calculated for each subject based on actual collection times and presented using summary statistics.

The absolute bioavailability will be calculated using the ratio of the insulin lispro AUC(0-∞) following injection into the thigh, abdomen, and deltoid to the insulin lispro AUC(0-∞)

following IV administration. A table of these percent absolute bioavailabilities will be presented.

10.3.2.2. Pharmacokinetic Statistical Inference

Log-transformed AUC(0-∞) and C_{max} for insulin lispro will be evaluated to estimate geometric least-squares means (LSmeans), ratios of geometric LSmeans between injection sites (deltoid and thigh compared with the abdomen), and their corresponding 90% CIs using the statistical model that includes injection site, period, and sequence as fixed effects, and subject within sequence as a random effect. The primary parameter for the statistical analysis of the relative bioavailability between injection sites will be the AUC(0-∞).

The analyses will also be performed using the subset of the subjects who complete all treatment periods with evaluable data.

10.3.3. Glucodynamic Analyses

10.3.3.1. Glucodynamic Parameter Estimation

Glucodynamic assessments will be determined from the glucose clamp procedure, where the GIR over time will be used as a measure of insulin effect. Glucodynamic analyses will be conducted on those subjects who complete at least 1 clamp procedure.

A locally weighted scatterplot smoothing (LOESS) function will be applied to all individual GIR versus time profiles in each treatment group and age group using S-PLUS software (version 8.2). The fitted data for each subject will be used to calculate the following GD parameters: maximum GIR (R_{max}), time to R_{max} (tR_{max}), and total amount of glucose infused (G_{tot}). Additional GD parameters, may be computed as necessary. The values of these GD parameters will be summarized by treatment group through descriptive statistics. Mean LOESS fits of GIR versus time profiles will be generated.

10.3.3.2. Glucodynamic Statistical Inference

Log-transformed R_{max} and G_{tot} will be evaluated to estimate geometric LSmeans, ratios of geometric LSmeans between injection sites (deltoid and thigh compared with the abdomen), and their corresponding 90% CIs using the statistical model that includes injection site, period, and sequence as fixed effects, and subject within sequence as a random effect. For GD parameters that have at least 1 subject with a value equal to zero, a value equal to the smallest non-zero observed GD value for that parameter divided by 2 will be added to all values, and the analysis of the log-transformed data will be performed. In addition, as a sensitivity analysis, a nonparametric analysis will be performed for that parameter.

The analyses will also be performed using the subset of the subjects who complete all treatment periods with evaluable data.

10.3.4. Pharmacokinetic/Pharmacodynamic Analyses

Not applicable.

10.3.5. Evaluation of Immunogenicity

The frequency of antibody formation to insulin lispro will be determined. The relationship between the presence (or absence) of antibodies and AEs will be assessed. Likewise, the relationship between the presence of antibodies and the PK parameters and GD response to insulin lispro may be assessed.

10.3.6. Data Review during the Study

This section is not applicable for this study.

10.3.7. Interim Analyses

No interim analyses are planned for this study. If an unplanned interim analysis is deemed necessary, the Lilly CP, CRP/investigator, or designee will consult with the appropriate medical director or designee to determine if it is necessary to amend the protocol.

11. References

Humalog [package insert]. Indianapolis, IN 46285: Lilly USA, LLC; 2015.

Remodulin [package insert]. Research Triangle Park, NC 27709: United Therapeutics Corp.; 2014.

Appendix 1. Abbreviations and Definitions

Term	Definition
AE	adverse event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.
AUC	area under the concentration versus time curve
AUC(0-10h)	area under the concentration versus time curve from time zero to 10 hours
AUC(0-tlast)	area under the concentration versus time curve from time zero to t_{last} where t_{last} is the last time point with a measurable concentration
AUC(0-∞)	area under the concentration versus time curve from time zero to infinity
BMI	body mass index
CI	confidence interval
CL	total body clearance of drug calculated after intravenous administration
CL/F	apparent total body clearance of drug calculated after extra-vascular administration
C_{max}	maximum observed serum concentration
compliance	Adherence to all the study-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
CP	clinical pharmacologist
CRF	case report form
CRP	clinical research physician: Individual responsible for the medical conduct of the study. Responsibilities of the CRP may be performed by a physician, clinical research scientist, global safety physician, or other medical officer.
CRU	clinical research unit
ECG	electrocardiogram
eCRF	electronic case report form
ERB	ethical review board
FBG	fasting blood glucose
FDA	Food and Drug Administration
GCP	good clinical practice
GD	glucodynamic(s)

GIR	glucose infusion rate
G_{tot}	total amount of glucose infused
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
informed consent	A process by which a subject voluntarily confirms his or her willingness to participate in a particular study, after having been informed of all aspects of the study that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form.
interim analysis	An interim analysis is an analysis of clinical study data, separated into treatment groups, that is conducted before the final reporting database is created/locked.
investigational product (IP)	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical study, including products already on the market when used or assembled (formulated or packaged) in a way different from the authorized form, or marketed products used for an unauthorized indication, or marketed products used to gain further information about the authorized form.
investigator	A person responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the investigator is the responsible leader of the team and may be called the principal investigator.
IV	Intravenous
LOESS	locally weighted scatterplot smoothing
LLOQ	lower limit of quantitation
LSmeans	least-squares means
MDI	multiple daily injection
open-label	A study in which there are no restrictions on knowledge of treatment allocation, therefore the investigator and the study participant are aware of the drug therapy received during the study.
PAH	pulmonary arterial hypertension
PK	pharmacokinetic(s)
randomize	The process of assigning subjects to an experimental group on a random basis.
R_{max}	maximum glucose infusion rate
SAE	serious adverse event

SC	Subcutaneous
screen	The act of determining if an individual meets minimum requirements to become part of a pool of potential candidates for participation in a clinical study.
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	half-life associated with the terminal rate constant in noncompartmental analysis
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TEAE	treatment-emergent adverse event: Any untoward medical occurrence that emerges during a defined treatment period, having been absent pretreatment, or worsens relative to the pretreatment state, and does not necessarily have to have a causal relationship with this treatment
t_{max}	time to maximum concentration
tR_{max}	time to R_{max}
V	volume of distribution
V/F	apparent volume of distribution after extravascular administration

Appendix 2. Clinical Laboratory Tests

Safety Laboratory Tests

Hematology[a]
- Hematocrit
- Hemoglobin
- Erythrocyte count (RBC)
- Mean cell volume
- Mean cell hemoglobin
- Mean cell hemoglobin concentration
- Leukocytes (WBC)
- Absolute counts of
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Platelets

Urinalysis[a]
- Specific gravity
- Ph
- Protein
- Glucose
- Ketones
- Bilirubin
- Urobilinogen
- Blood
- Nitrite
- Leukocytes
- Microscopy[e]

Clinical Chemistry[a]
- Sodium
- Potassium
- Bicarbonate
- Chloride
- Calcium (total)
- Calcium (ionized)
- Magnesium
- Phosphate
- Glucose
- Blood urea
- Total protein
- Albumin
- Total bilirubin
- Alkaline phosphatase (ALP)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Creatinine

Serology[b]
- Hepatitis B surface antigen
- Hepatitis C antibody
- HIV

Pregnancy Tests
- Pregnancy test[c]
- FSH[d]

Abbreviations: FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; RBC = red blood cell; WBC = white blood cell.

[a] Performed at screening, Predose Day 1 Period 1, 1 hour postdose (IV period) and follow-up. Fasting clinical laboratory tests at screening and Predose Day 1 Period 1.

[b] Only at screening. This test will not be performed if results of the same test have been obtained from the subject within the past 6 months.

[c] A blood test for pregnancy will be done at screening, and a urine pregnancy test will be done at times indicated in the Schedule of Activities (Section 2).

[d] Only at screening in women for assessment of menopause status if necessary.

[e] If clinically indicated, per investigator's discretion.

Note: Results of these assays will be validated by the local laboratory at the time of testing. Additional tests may be performed or auto-calculated by the laboratory as part of its standard panel that cannot be removed. Some of the above parameters are calculated from measured values. Omission of calculated values will not be considered as a protocol violation.

Appendix 3. Study Governance, Regulatory, and Ethical Considerations

Informed Consent

The investigator is responsible for

- ensuring that the subject understands the nature of the study, the potential risks and benefits of participating in the study, and that their participation is voluntary.
- ensuring that informed consent is given by each subject or legal representative. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of IP.
- answering any questions the subject may have throughout the study and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the study.
- providing a copy of the ICF to the participant or the participant's legal representative and retaining a copy on file.

Recruitment

Study-specific recruitment material should be approved by Lilly.

Ethical Review

The investigator or appropriate local representative must give assurance that the ERB was properly constituted and convened as required by International Council for Harmonisation (ICH) guidelines and other applicable laws and regulations.

Documentation of ERB approval of the protocol and the ICF must be provided to Lilly before the study may begin at the CRU. Lilly or its representatives must approve the ICF before it is used at the CRU. All ICFs must be compliant with the ICH guidelines on GCP.

The CRU's ERB(s) should be provided with the following:

- the current IB and updates during the course of the study
- ICF
- relevant curricula vitae

Regulatory Considerations

This study will be conducted in accordance with the protocol and with

1) consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
2) applicable ICH GCP guidelines

3) applicable laws and regulations

Some of the obligations of the sponsor will be assigned to a third-party organization.

Protocol Signatures

The sponsor's responsible medical officer will approve the protocol, confirming that, to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, the principal investigator will sign the protocol signature page and send a copy of the signed page to a Lilly representative.

Final Report Signature

The investigator or designee will sign the clinical study report for this study, indicating agreement with the analyses, results, and conclusions of the report.

The sponsor's responsible medical officer and statistician will sign/approve the final clinical study report for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

Data Quality Assurance

To ensure accurate, complete, and reliable data, Lilly or its representatives will do the following:

- provide instructional material to the study sites, as appropriate.
- provide training to instruct the investigators and study coordinators. This training will give instruction on the protocol, the completion of the CRFs, and study procedures.
- make periodic visits to the study site.
- be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
- review and evaluate CRF data and/or use standard computer edits to detect errors in data collection.
- conduct a quality review of the database.

In addition, Lilly or its representatives will periodically check a sample of the subject data recorded against source documents at the CRU. The study may be audited by Lilly and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

The investigator will keep records of all original source data. This might include laboratory tests, medical records, and clinical notes. If requested, the investigator will provide the sponsor, applicable regulatory agencies, and applicable ERBs with direct access to the original source documents.

Data Collection Tools/Source Data

An electronic data capture system will be used in this study. The CRU must define and retain all source records and must maintain a record of any data where source data are directly entered into the data capture system.

Data Protection

Data systems used for the study will have controls and requirements in accordance with local data protection law.

The purpose and use of subject personal information collected will be provided in a written document to the subject by the sponsor.

Study and Site Closure

Discontinuation of Study Sites

Clinical research unit participation may be discontinued if Lilly, the investigator, or the ERB of the CRU judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Discontinuation of the Study

The study will be discontinued if Lilly judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Appendix 4. Blood Sampling Summary

This table summarizes the approximate number of venipunctures and blood volumes for all blood sampling (screening, safety laboratories, and bioanalytical assays) during the study.

Protocol I8B-MC-ITRT Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests[a,b]	17	1	17
Clinical laboratory tests[a,b]	1×11 (fasting)	1	29
	1×9 (nonfasting)	2	
Pharmacokinetic samples serum insulin lispro	1	22 samples × 4 periods = 88	88
Pharmacokinetic samples plasma treprostinil	2	4 samples × 1 period = 4	8
C-peptide samples	2	12 samples × 4 periods = 48	96
Blood for glucose	0.2	76 samples × 4 periods = 304	60.8
Blood discard for cannula patency	0.25	76 samples × 4 periods = 304	76
Immunogenicity	5	3	15
Pharmacogenetics	10	1	10
Total for clinical purposes			399.8
Total for clinical purposes rounded up to the nearest 10 mL			400

a Additional samples may be drawn if needed for safety purposes.

b Fasting clinical laboratory tests at screening, and Predose Day 1 Period 1.

Appendix 5. Protocol Amendment I8B-MC-ITRT(a) Summary
Effect of Injection Site on the Relative and Absolute Bioavailability of Single Dose of LY900014 in Healthy Subjects

Overview

Protocol I8B-MC-ITRT [Effect of Injection Site on the Relative and Absolute Bioavailability of Single Dose of LY900014 in Healthy Subjects] has been amended. The new protocol is indicated by Amendment (a) and will be used to conduct the study in place of any preceding version of the protocol.

The overall changes and rationale for the changes made to this protocol are as follows:

- The sample size was adjusted to ensure adequate precision to estimate the 90% CI for the ratio for SC injection into the thigh and deltoid, compared to the abdomen.
- The arm injection site was changed to deltoid to ensure consistency in protocol language.
- PK and GD parameters (time to early half-maximal plasma concentration [early 50% t_{max}], time to late half-maximal drug concentration [late 50% t_{max}], time to half-maximal GIR before tR_{max} [early 50% tR_{max}], time to half-maximal GIR after tR_{max} [late 50% tR_{max}], time to onset of insulin action [T_{onset}], and G_{tot} over 10 hours [$Gtot_{[0\text{-}10h]}$], partial glucose AUCs, such as total amount of glucose infused over 30 minutes, 1 or 2 hours) were removed as they were not required for the statistical analysis to support the objectives of the study. There is no change to primary and secondary endpoints.
- The criterion to include subjects with body weight ≥45 kg and hemoglobin level of ≥12.5 g/dL at screening was included based on recommendation of Domain Specific Review Board (DSRB), Singapore. The rationale given was that a blood volume of approximately 400 mL or more would be withdrawn per subject throughout the study.

Revised Protocol Sections

Note:	All deletions have been identified by ~~strikethroughs~~. All additions have been identified by the use of underscore.

The numbering system used for inclusion and exclusion criteria provides a unique number for each criterion and allows for efficiency in data collection.

In case an amendment to the protocol adds a criterion, that criterion will receive the next available number, regardless of whether it is an inclusion or exclusion criterion.

1 Protocol Synopsis

Rationale:
The primary objective of this study is therefore to compare the bioavailability of insulin lispro following administration of the commercial formulation of LY900014 at 3 different injection sites SC in the abdominal wall, thigh, and ~~arm~~ deltoid. In addition, the intravenous (IV) administration will serve as the reference to determine the absolute bioavailability of SC injections into the ~~arm~~ deltoid, thigh or abdomen and GD activity.

Number of Subjects:
Up to ~~24~~ 28 healthy men and women may be enrolled to target at least ~~16~~ 22 subjects complete the study.

3.1 Study Rationale

The primary objective of this study is therefore to compare the bioavailability of insulin lispro following administration of the commercial formulation of LY900014 at 3 different injection sites SC in the abdominal wall, thigh, and ~~arm~~ deltoid. In addition, the intravenous (IV) administration will serve as the reference to determine the absolute bioavailability of SC injections into the ~~arm~~ deltoid, thigh, or abdomen and GD activity.

5.1 Overall Design

This is a Phase 1, single-center, open-label, 4-period, randomized, crossover, up to 10-hour euglycemic clamp study in approximately ~~24~~28 healthy subjects to compare the insulin lispro PK and GD of LY900014 after SC administration of a 15-U dose at 3 different injection sites and a single IV bolus injection of 15 U.

5.2 Number of Participants

A total of ~~24~~ 28 subjects may be enrolled so that at least ~~16~~ 22 subjects complete the study.

If subjects discontinue from the study before completion of all 4 study periods, replacement subjects may be enrolled up to ~~24~~ 28 subjects following agreement between the investigator and the sponsor.

6.1 Inclusion Criteria

[3] have a body weight of ≥45 kg, and body mass index (BMI) of 18.0 to 30.0 kg/m^2, inclusive

6.2 Exclusion Criteria

Additional inclusion criterion:

[27] have a hemoglobin level of ≥12.5 g/dL at screening

10.1. Sample Size Determination

Up to ~~24~~ 28 subjects may be enrolled to ensure that at least ~~16~~ 22 subjects complete the study. ~~Sixteen~~ Twenty-two completing subjects will provide ~~at least 90% power to show the~~ estimated 2-sided 90% confidence intervals (CIs) of the ratios of geometric means for ~~area under the concentration versus time curve (AUC) from time zero to infinity (~~AUC[0-∞]~~)~~ after SC injection into the thigh and deltoid, compared to the abdomen, to be within approximately ~~20% of the geometric mean ratio estimate~~ 0.8 to 1.25 when the observed ratio is 1. This calculation is based on the assumption of a log-normal distribution and an estimate of intrasubject log-scale standard deviation of 0.2. ~~There is also approximately 86% power for the comparisons between injection sites for maximum observed serum concentration (C_{max}), assuming an intrasubject log-scale standard deviation of 0.35.~~

10.3.2.1 Pharmacokinetic Parameter Estimation

Free serum insulin lispro concentrations will be used to calculate several PK parameters, including maximum observed drug concentration (C_{max}), time of maximum observed drug concentration (t_{max}), $t_{1/2}$, ~~time to early half-maximal plasma concentration (early 50% t_{max}), time to late half-maximal drug concentration (late 50% t_{max}), and~~ area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration (AUC[0-tlast]), AUC from time zero to 10 hours (AUC[0-10h]), area under the concentration versus time curve from time zero to infinity (AUC[0-∞]), apparent total body clearance of drug calculated after extra-vascular administration (CL/F), and apparent volume of distribution during the terminal phase after extra-vascular administration (Vz/F) will be determined.

10.3.3.1 Glucodynamic Parameter Estimation

A locally weighted scatterplot smoothing (LOESS) function will be applied to all individual GIR versus time profiles in each treatment group and age group using S-PLUS software (version 8.2). The fitted data for each subject will be used to calculate the following GD parameters: maximum GIR (R_{max}), time to R_{max} (tR_{max}), ~~time to half-maximal GIR before tR_{max} (early 50% tR_{max}), time to half-maximal GIR after tR_{max} (late 50% tR_{max}), time to onset of insulin action (T_{onset}),~~ and total amount of glucose infused (G_{tot}), ~~and G_{tot} over 10 hours ($Gtot_{[0\text{-}10h]}$).~~ Additional GD parameters ~~partial glucose AUCs, such as total amount of glucose infused over 30 minutes, 1 or 2 hours~~ may be computed as necessary. The values of these GD parameters will

be summarized by treatment group through descriptive statistics. Mean LOESS fits of GIR versus time profiles will be generated.

Leo Document ID = 3d691570-b5ac-4e8d-ac55-5ebcfe66512e

Approver: PPD
Approval Date & Time: 14-Jul-2017 12:38:59 GMT
Signature meaning: Approved

Approver: PPD
Approval Date & Time: 14-Jul-2017 13:11:04 GMT
Signature meaning: Approved