Protocol B5K-EW-IBHG(b)

Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus

NCT02588950

Approval Date: 07-Jun-2016

1. Protocol B5K-EW-lBHG(b) Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus

Human Regular U-500 Insulin (LY041001)

A Phase 1 (postmarketing), single-center, 2-part, randomized, open-label, 3-period, 4-sequence, crossover-design euglycemic clamp study in high-dose insulin-treated subjects with type 2 diabetes mellitus to compare the pharmacokinetics (PK) and pharmacodynamics (PD) of a bolus of 100 U human regular U-500 insulin (U-500 R) administered by single subcutaneous injection compared to continuous subcutaneous insulin infusion (Part A). Part B will describe the PK and PD of U-500 R at steady state administered by multiple daily injections either TID or BID.

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Indianapolis, Indiana USA 46285

Protocol Electronically Signed and Approved by Lilly: 29 April 2015
Amendment (a) Electronically Signed and Approved by Lilly: 24 August 2015
Amendment (b) Electronically Signed and Approved on approval date provided below:

Approval Date: 07-Jun-2016 GMT

2. Synopsis

Clinical Pharmacology Protocol Synopsis: Study B5K-EW-IBHG

Name of Investigational Product: Human regular U-500 insulin (LY041001)	
Title of Study: Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus	
Number of Planned Subjects: Up to 32 subjects may be enrolled to ensure approximately 24 complete the study	**Phase of Development:** 1 (postmarketing)
Length of Study: Ranging from approximately 7 to 13.5 weeks (first screening visit to last follow-up visit)	
Objectives: The primary objective of Part A is to compare the time of maximum drug concentration (t_{max}) of a 100-U bolus of human regular U-500 insulin (U-500R) (500 U/mL) administered via single subcutaneous (SC) injection versus a 100-U bolus of U-500R administered via continuous SC insulin infusion (CSII) in high-dose insulin-treated subjects with type 2 diabetes mellitus (T2DM). The primary objective of Part B is to evaluate the area under the concentration versus time curve (AUC) from time zero to 24 hours postdose (AUC[0-24 hr]), maximum drug concentration (C_{max}), and maximum glucose infusion rate (GIR) (R_{max}) of U-500R at steady state following thrice- (TID) or twice- (BID) daily SC dosing in the study population. The secondary objective of Part A is to compare the time to maximum glucose infusion rate (tR_{max}) and exposure (AUC from time zero to time t, where t is the last time point with a measurable concentration [AUC(0-t_{last})]) of a 100-U bolus of U-500R administered via single SC injection versus CSII in the study population. The secondary objective of Part B is to evaluate t_{max}, total amount of glucose infused (G_{tot}), and the time of maximum GIR (tR_{max}) of U-500R at steady state following TID and BID SC dosing in the study population. Exploratory objectives are to evaluate other pharmacokinetic (PK) and pharmacodynamic (PD) parameters including, but not limited to half-life associated with the terminal rate constant in noncompartmental analysis ($t_{1/2}$), 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 (V_z/F), the time of last GIR measurement (tR_{last}), the time of first change of GIR postdose (tR_{onset}), the time of 50% of maximum GIR before R_{max} (early tR_{max50}) , and the time of 50% of maximum GIR after R_{max} (late tR_{max50}) in the study population in Part A, and AUC of U-500R over each dosing interval and over each meal at steady state following TID or BID SC dosing in the study population in Part B.	
Study Design: This is a Phase 1 (postmarketing), single-center, 2-part, 3-period, 4-sequence, randomized, open-label, crossover design study. In Part A, subjects will receive 100-U bolus of U-500R by SC injection in 1 period and by CSII with basal infusion in another period (with sequence specified by the randomization schedule), prior to undergoing a euglycemic clamp procedure with a maximum duration of 24 hours. In Part B, subjects will be randomized to receive U-500R either TID or BID for 5 to 10 days prior to undergoing a 24-hour euglycemic clamp procedure.	
Diagnosis and Main Criteria for Inclusion and Exclusion: High-dose insulin-treated (U-100, U-200, and/or U-300 insulin total daily dose [TDD] ≥150 U/day OR at least 1 dose >100 U as part of a multiple daily injection (MDI) regimen AND have TDD ≤3.0 U/kg) males or females with T2DM, ≥18 to <75 years old, with a body mass index of ≥27.0 kg/m^2, and HbA1c 7.5% to 11.5%, inclusive, at screening.	
Test Product, Dosage, and Mode of Administration: Human regular U-500 insulin. Part A: 100 U, given as bolus once by single SC injection in 1 period and once by CSII in another period (following a 12-hour 4.25-U/hour infusion started 12 hours prior to the CSII bolus and continuing throughout the clamp). Part B: Individually tailored doses given either TID or BID by SC injection in the 5 to 10 days prior to and during Period 3. Randomized sequences will be followed for Parts A and B.	
Comparator, Dose, and Mode of Administration: None.	

Planned Duration of Treatment: Screening to occur up to 28 days prior to Period 1. Part A: 100 U bolus of U-500R will be administered once in each of 2 periods with 7 to 21 days between doses. Part B: U-500R will be administered TID or BID for up to 12 days. Each period will take approximately 2 days. Follow-up will occur 7 to 28 days after the end of the Period 3 clamp. Total maximum study duration approximately 13.5 weeks.

Criteria for Evaluation:

Safety: Safety data will include recording of adverse events, hypoglycemic events, self-monitored plasma glucose, clinical laboratory evaluations, vital signs, 12-lead electrocardiograms, body weight, and physical examinations.

Bioanalytical: Whole blood samples will be collected and analyzed immediately for blood glucose (BG) concentration by the euglycemic clamp device/method. Blood samples will be collected for the determination of serum immunoreactive insulin and C-peptide concentrations for up to 24 hours after dose administration in Part A and for 24 hours in Part B.

Pharmacokinetic/Pharmacodynamic: Pharmacokinetic parameters will be evaluated by standard noncompartmental methods of PK analysis. The primary PK parameter in Part A will be t_{max}. Other noncompartmental parameters such as C_{max}, AUC from time zero to infinity (AUC[0-∞]), AUC(0-t_{last}), $t_{1/2}$, CL/F, and V_z/F may be reported, as appropriate. The primary PK parameters for Part B will be AUC(0-24) and C_{max}; other parameters such as t_{max} and AUC of U-500R over each dosing interval and over each meal at steady state may also be reported, as appropriate.

Pharmacodynamic parameters will be derived from the glucose clamp data. Blood samples will be obtained during the euglycemic glucose clamp procedure for analysis of whole BG. Glucose infusion rates will be adjusted to maintain euglycemia with the documented GIRs over time, providing the primary PD measure of insulin action. Glucose infusion rates will be used to calculate several PD parameters in each part of the study, including G_{tot}, R_{max}, tR_{max}, and tR_{last}. Other parameters, such as tR_{onset}, early tR_{max50}, and late tR_{max50}, may be reported. Parameters will be individually calculated for each subject and presented by summary statistics.

Statistical Evaluation Methods:

Safety: The parameters will be listed and summarized using standard descriptive statistics as appropriate. Additional analysis will be performed if warranted upon review of the data.

Pharmacokinetic/Pharmacodynamic: For Part A, the primary parameter for statistical analysis will be t_{max}. The values for t_{max} will be log-transformed and evaluated in a linear mixed-effects model with fixed effects for administration method, period and sequence (sequences 1 and 3, versus 2 and 4), and a random effect for subject. The difference in least squares means along with the 90% confidence interval (CI) will be back-transformed to present the ratios of geometric means and the CI for comparison of the administration methods (single SC injection and CSII). This same analysis will be repeated for AUC(0-tlast), AUC(0-∞), and $C_{max,}$ and the PD parameters (R_{max} and G_{tot}).

The values for t_{max} will also be analyzed non-parametrically using a Wilcoxon signed-rank test. Median differences and approximate 90% CIs for the difference will be calculated for comparison of the administration methods (single SC injection and CSII).

For Part A, the values of tR_{max} will be analyzed non-parametrically using a Wilcoxon signed-rank test as described above. Other PD time parameters will be analyzed non-parametrically.

Exploratory analyses may be performed for other PK and PD parameters as deemed appropriate.

Descriptive statistics will be reported for other PK and PD parameters in Part A and all PK and PD parameters in Part B.

3. Table of Contents

Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus

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4. Abbreviations and Definitions

Term	Definition
ADA	American Diabetes Association
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.
AHA	antihyperglycemic agent
AUC	area under the concentration versus time curve
AUC(0-∞)	area under the concentration versus time curve from time zero to infinity
AUC(0-24)	area under the concentration versus time curve from time zero to 24 hours postdose
AUC(0-t_{last})	area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration
BG	blood glucose
BID	twice daily
BMI	body mass index
CI	confidence interval
CL/F	apparent total body clearance of drug calculated after extra-vascular administration
C_{max}	maximum drug concentration
complaint	A complaint is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, purity, durability, reliability, safety or effectiveness, or performance of a drug or drug delivery system.
compliance	Adherence to all the trial-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
CRF/eCRF	case report form/electronic case report form: Sometimes referred to as clinical report form. A printed or electronic form for recording study participants' data during a clinical study, as required by the protocol.
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

CSE	clinically significant event: A moderate to severe adverse event (AE), abnormal clinical sign, or clinical laboratory finding that may pose a risk to the well-being of the subject.
CSII	continuous subcutaneous insulin infusion
early tR_{max50}	time of 50% of maximum glucose infusion rate (GIR) before maximum GIR (R_{max})
ECG	electrocardiogram
end of trial (study)	End of trial is the date of the last visit or last scheduled procedure shown in the Study Schedule for the last subject.
enroll	The act of assigning a subject to a treatment. Subjects who are enrolled in the trial are those who have been assigned to a treatment.
enter	Subjects entered into a trial are those who sign the informed consent form directly or through their legally acceptable representatives.
ERB/IRB	ethical review board/institutional review board: A board or committee (institutional, regional, or national) composed of medical professionals and nonmedical members whose responsibility is to verify that the safety, welfare, and human rights of the subjects participating in a clinical trial are protected.
FSH	follicle-stimulating hormone
GCP	good clinical practice: A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.
GIR	glucose infusion rate
G_{tot}	total amount of glucose infused
HbA1c	hemoglobin A1c
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonisation
informed consent	A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial 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 trial data, separated into treatment groups, that is conducted before the final reporting database is created/locked.

IP	investigational product: A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, 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 trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
IRI	immunoreactive insulin
late tR_{max50}	time of 50% of maximum glucose infusion rate (GIR) after R_{max}
legal representative	An individual or judicial or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.
MET	metformin
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.
PD	pharmacodynamic(s)
PG	plasma glucose
PK	pharmacokinetic(s)
QTc	corrected QT interval
randomize	The process of assigning subjects to an experimental group according to the randomization schedule for the trial.
re-screen	To screen a subject who was previously declared a screen failure for the same study.
R_{max}	maximum GIR
SAE	serious adverse event: Any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.
SC	subcutaneous(ly)
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 trial. In this study, screening involves invasive or diagnostic procedures and/or tests (such as blood draws). For this type of screening, informed consent for these screening procedures and/or tests shall be obtained; this consent may be separate from obtaining consent for the study.

SGLT2	sodium-glucose co-transporter-2
SMPG	self-monitored plasma glucose
subject	A study participant who has the disease or condition for which the investigational product is targeted.
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
TDD	total daily dose
TID	thrice daily
t_{max}	time of C_{max}
TPO	third party organization
tR_{last}	time of last glucose infusion rate (GIR) measurement
tR_{max}	time of maximum glucose infusion rate (GIR)
tR_{onset}	time of first change of glucose infusion rate (GIR) postdose
U-100R	human regular U-100 insulin
U-500R	human regular U-500 insulin
U-500 OmniPod	U-500 OmniPod Insulin Management System
USPI	United States package insert
V_z/F	apparent volume of distribution during the terminal phase after extra-vascular administration
WHO	World Health Organization

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5. Introduction

5.1. General Introduction

As obesity and insulin resistance become more prevalent, insulin dose requirements in diabetes mellitus patients are rising (Segal et al. 2010). Patients treated with high doses (and therefore high volumes) of U-100 insulin may require up to 8 or more daily injections. The use of concentrated human regular U-500 insulin (U-500R; 500 U/mL) allows a large dose to be administered in one-fifth the volume as compared to the U-100 concentration (100 U/mL) (Humulin-R U-500 Package Insert, 2014). Accordingly, there has been a steady increase in the number of U-500R prescriptions written per month in recent years (Lane et al. 2009; Segal et al. 2010; de la Peña et al. 2011).

Human regular U-500 insulin is indicated for use in the treatment of severely insulin-resistant patients with diabetes requiring insulin daily doses >200 U. The United States package insert (USPI) advises administration via subcutaneous (SC) injection and states that Humulin-R U-500 is usually given 2 or 3 times daily before meals. However, a recent real-world large U.S. database study reported that over a quarter of patients treated with U-500R receive it via off-label continuous SC insulin infusion (CSII) (Eby et al. 2014). A review and meta-analysis of case series of patients treated with U-500R by Reutrakul et al. (2012) reported on outcomes in 55 patients who used U-500R via CSII. In the mean follow-up time of 3 to 30 months after initiation of U-500R via CSII, hemoglobin A1c (HbA1c) significantly decreased by a mean of 1.64%, which was similar to the findings with U-500R administered by multiple daily injections (MDIs) (mean decrease in HbA1c of 1.59%). Patients treated with U-500R CSII achieved glycemic improvement with a nonsignificant decrease in total daily dose (TDD) of 13.6 U from U-100 insulin dose at baseline, in contrast to the MDI patients who had a statistically significant mean increase in TDD of 51.9 U from baseline U-100 insulin dose.

Clinical experience has shown that a single SC dose of U-500R frequently has time-action characteristics reflecting both prandial and basal activity. It takes effect within 30 minutes, has a peak similar to that observed with human regular U-100 insulin (U-100R), and has a relatively long duration of activity (up to 24 hours) following a single dose as compared with U-100R. This effect has been attributed to the high concentration of the preparation (Humulin-R U-500 package insert, 2014).

In healthy obese subjects with mean body mass index (BMI) 34.4 ± 2.6 kg/m^2 (Study B5K-EW-IBHA; de la Peña et al. 2011), U-500R administered by single SC injection as

50- or 100-U doses (0.4 to 0.6 and 0.8 to 1.3 U/kg, respectively) resulted in similar overall exposure (area under the concentration versus time curve [AUC] from time zero to return to baseline) to that observed after administration of equal doses of U-100R, although maximum observed drug concentrations (C_{max}) were lower. Findings in the pharmacodynamic (PD) parameters generally reflected the pharmacokinetic (PK) findings, with similar overall effect (total amount of glucose infused [G_{tot}]) and lower maximum effect (maximum glucose infusion rate [R_{max}]). Time of maximum drug concentration (t_{max}) and the time of maximum effect (time of maximum glucose infusion rate [tR_{max}]) were significantly longer for U-500R compared to U-100R at only the 100-U dose. Duration of action (time of last glucose infusion rate measurement [tR_{last}]) and effect after the peak (time of 50% of maximum glucose infusion rate after R_{max} [late tR_{max50}]) were significantly prolonged for U-500R versus U-100R at both doses.

The only published PK study of U-500R in obese patients with type 2 diabetes mellitus (T2DM) with severe insulin resistance was a small non-clamp study conducted by Davidson et al. (2010). Nine severely insulin-resistant obese patients with T2DM and poor glycemic control were given SC boluses of 100 U of U-500R. Onset of action took place in 30 minutes or less with peak insulin levels at 5 hours and persistent elevation of insulin concentration was observed 7 hours postdose. The minimum time to reach a target plasma glucose (PG) level of less than 100 mg/dL (5.6 mmol/L) ranged from 3 to more than 7 hours (Davidson et al. 2010).

Recently, in order to predict steady-state PK/PD of high-dose U-500R, de la Peña et al. (2014) conducted a modeling and simulation study. The model was built from 3 single-dose, crossover, euglycemic clamp PK/PD studies with human regular insulin. Healthy obese (de la Peña et al. 2011) and healthy normal-weight subjects, and patients of normal weight with type 1 diabetes mellitus (T1DM) participated in the studies. A 1-compartment, first-order absorption and elimination PK model was used and provided independent absorption rate constants for both U-500R and U-100R. An effect compartment model was used to describe the PD parameters (maximum pharmacologic effect [E_{max}] and concentration to achieve 50% E_{max}). Human regular U-500 insulin PK/PD simulation profiles were performed for once-daily, twice-daily (BID), and thrice-daily (TID) dosing regimens (de la Peña et al. 2014).

All 3 dosing regimens were predicted to achieve steady state PK by 24 hours after the initial insulin dose. At steady state, the greatest fluctuations in PK/PD parameters were observed with once-daily dosing. Conversely, BID dosing resulted in an increase in insulin concentration and effect with each dose and a stable basal effect while TID dosing resulted in sustained effects between doses. Similar to the findings of de la Peña et al. (2011), the U-500R PK/PD simulation modeling study indicated the steady state basal PD effect from both BID and TID dosing appears to support use of U-500R as insulin monotherapy, without the need for concomitant U-100 basal insulin (de la Peña et al. 2014).

Information on serious adverse events (SAEs) expected in the study population independent of drug exposure and that will be assessed by the sponsor in aggregate, periodically during the course of the study, may be found in the USPI.

5.2. Rationale and Justification for the Study

There have been no state-of-the-art euglycemic clamp PK/PD studies performed on U-500R in the target population of high-dose insulin-treated subjects with T2DM, in whom U-500R is most often used (Lane et al. 2009; Reutrakul et al. 2012; Jones and Idris 2013; Eby et al. 2013; Eby et al. 2014; Cochran et al. 2014). Likewise, there have been no PK/PD studies conducted on steady state time-concentration and time-action characteristics of U-500R on any population of subjects. Rigorous study in both of these settings will address major gaps in scientific knowledge and will further inform clinicians regarding appropriate use of U-500R in insulin-resistant patients with diabetes requiring high doses of insulin.

Although the findings of studies describing clinical outcomes of off-label treatment with U-500R by CSII in patients appear favorable (Lane 2006; Lane et al. 2009; Lane et al. 2010; Reutrakul et al. 2012; Jones and Idris 2013; Lane et al. 2013), there are no PK/PD data available regarding bolus dosing via this method of administration. Dramatic improvements in continuous glucose monitoring patterns and flattening of postprandial PG excursions have been observed after 13 to 52 weeks of high-dose U-500R therapy (Lane et al. 2010). This raises the possibility that U-500R via CSII may have a more rapid onset and perhaps greater prandial effects as compared to that observed by de la Peña et al. (2011) for SC bolus injections of U-500R, and by Hood et al. (2015) in the recent 24-week randomized clinical trial of U-500R comparing TID with BID dosing algorithms in severely insulin-resistant patients with poorly controlled T2DM.

Part A of this study seeks to evaluate and compare the PK and PD characteristics of a 100-U insulin dose using U-500R insulin administered via standard SC injection versus bolus administration delivered via CSII, with the primary measure being t_{max}. Part B of the study will evaluate the steady state PK/PD of U-500R administered TID and BID in the target population, thus supplementing current PK/PD data obtained from studies in healthy and obese subjects and enabling validation of predictions made through PK/PD modeling.

6. Objectives

6.1. Primary Objective

Part A: to compare the t_{max} of a 100-U bolus of U-500R (500 U/mL) administered via single SC injection versus a 100-U bolus of U-500R administered via CSII in high-dose insulin-treated subjects with T2DM.

Part B: to evaluate the AUC from time zero to 24 hours postdose (AUC[0-24]), C_{max}, and R_{max} of U-500R at steady state following TID or BID SC dosing in the study population.

6.2. Secondary Objective

Part A: to compare the tR_{max} and exposure (AUC from time zero to time t, where t is the last time point with a measurable concentration [AUC(0-t_{last})]) of a 100-U bolus of U-500R administered via single SC injection versus CSII in the study population.

Part B: to evaluate the t_{max}, G_{tot}, and tR_{max} of U-500R at steady state following TID or BID SC dosing in the study population.

6.3. Exploratory Objective

Other PK and PD parameters may be evaluated including but not limited to:

Part A: half-life associated with the terminal rate constant in noncompartmental analysis ($t_{1/2}$), 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 (V_Z/F), tR_{last}, the time of first change of GIR postdose (tR_{onset}), the time of 50% of maximum GIR before R_{max} (early tR_{max50}), and late tR_{max50} in the study population.

Part B: AUC of U-500R over each dosing interval and over each meal at steady state following TID or BID SC dosing in the study population.

7. Investigational Plan

7.1. Summary of Study Design

This is a 2-part, Phase 1 (postmarketing), single-center, 3-period, 4-sequence, randomized, open-label, crossover euglycemic clamp study in high-dose insulin-treated subjects with T2DM. Part A (Periods 1 and 2) will compare the PK and PD of a 100-U bolus of U-500R administered via single SC injection versus a 100-U bolus of U-500R administered via CSII. Subjects completing Part A will transition to Part B (Period 3), which will evaluate the PK and PD characteristics of U-500R administered by TID and BID MDIs under steady-state conditions.

Up to 32 subjects may be enrolled to achieve a target of approximately 24 completing all 3 periods of the study.

Subjects will be randomly assigned to 1 of 4 dosing sequences (Table IBHG.7.1). Each subject will be administered 100 U of U-500R by single SC injection, and 100 U of U-500R by CSII in Part A and either TID U-500R or BID U-500R in Part B.

Table IBHG.7.1. Dosing Schedule

	Part A		Part B
Sequence	Period 1	Period 2	Period 3
1	SC	CSII	TID
2	CSII	SC	BID
3	SC	CSII	BID
4	CSII	SC	TID

Abbreviations: BID = twice-daily administration of U-500R; CSII = 100 U U-500R administered via continuous subcutaneous insulin infusion; SC = 100 U U-500R administered via single subcutaneous injection; TID = thrice-daily administration of U-500R.

The study design is illustrated in Figure IBHG.7.1.

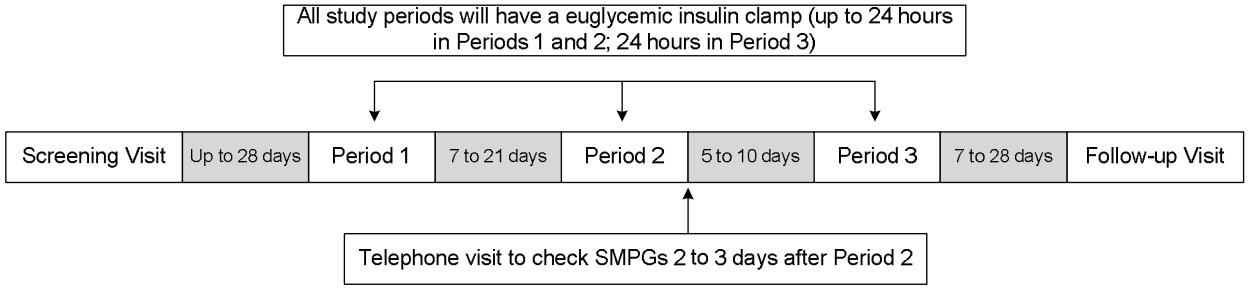

Figure IBHG.7.1. IBHG study design

7.1.1. Screening Visit

Subjects meeting all of the inclusion criteria and none of the exclusion criteria will be enrolled into the study. The screening visit may occur up to 28 days before Day 1 of Period 1.

7.1.2. Part A (Study Periods 1 and 2)

Subjects will be admitted to the clinical research unit (CRU) on Day -1 of each period and be fasted from 12 hours prior to clamp initiation until completion of the clamp procedure.

All subjects will stop their prestudy U-100, U-200, and/or U-300 insulin dosing at least 15 hours prior to U-500R bolus dosing. Subjects randomized to receive U-500R by SC injection will receive a 100-U U-500R bolus administration by SC injection prior to initiation of the clamp. Subjects randomized to receive U-500R by CSII will have a U-500 OmniPod Insulin Management System (U-500 OmniPod) fitted by a qualified member of CRU staff, which will administer a 12-hour infusion of 4.25 U/h of U-500R, prior to bolus administration of 100 U of U-500R (via the same pump) and initiation of the clamp. The 4.25-U/h U-500R infusion will continue throughout the duration of the clamp following bolus administration by the U-500 OmniPod.

Overnight, between Day -1 and Day 1, all subjects will have their PG stabilized, using an insulin lispro and/or glucose drip, to a target fasting PG concentration of 120 mg/dL (6.7 mmol/L) (with an acceptable range of 100 to 130 mg/dL [5.6 to 7.2 mmol/L]).

Following bolus dose administration (or initiation of bolus dose administration for CSII), subjects will undergo a euglycemic clamp with a maximum duration of 24 hours. Following completion of the clamp procedures, subjects will be provided a meal and will be medically assessed and discharged from the CRU, unless the investigator has any safety concerns. If glucose is still being infused at the time of clamp discontinuation, the subject will be monitored for safety and will remain in the CRU until stable according to investigator judgment (see Section 10.4.1).

Blood samples will be collected prior to and during the clamp procedure (see Attachment 1) to determine serum immunoreactive insulin (IRI) and C-peptide concentrations for PK and PD analysis.

Following termination of the clamp procedure in Period 1, subjects will resume their prestudy treatment regimen (which may include basal; premixed; and/or basal/bolus U-100, U-200, and/or U-300 insulin regimens with or without antihyperglycemic agents [AHAs] as listed in Inclusion Criterion [5], Section 8.1.1) until their admission to the CRU on Day -1 of Period 2. There will be a washout period of 7 to 21 days between U-500R bolus doses in Periods 1 and 2.

Following termination of the clamp procedure in Period 2, subjects will be instructed on administration of U-500R either by TID or BID MDIs as described in Section 7.1.3 and Section 9.4. There will be a period of 5 to 10 days between the end of the clamp in Period 2 and the clamp procedure in Period 3 to allow U-500R concentrations to reach steady state.

7.1.3. Part B (Study Period 3)

Following termination of the clamp procedure in Period 2, subjects will transition, as randomized, to TID or BID MDIs of U-500R. Human regular U-500 insulin will be self-administered by the subjects at home under telephone guidance from CRU staff during a telephone visit 2 to 3 days after the end of Period 2 (additional telephone or office visits may be added at the investigator's discretion). Initial U-500R doses will be based on each subject's insulin TDD prior to admission for Period 2, with titration for safety, if necessary, using the formulas given in Section 9.4. There will be 5 to 10 days between the end of Period 2 and the clamp in Period 3 (that is, dosing will begin on up to Day -10 and no later than Day -5 of Period 3) to allow steady state of the U-500R dosing regimen to be achieved. Mean self-monitored plasma glucose (SMPG) will be recorded from Day -5 until admission to the CRU.

Subjects will be admitted to the CRU on Day -1 of Period 3, prior to their pre-evening meal dose, which will be administered by CRU staff, and undergo fasting, PG stabilization, and a 24-hour euglycemic clamp as detailed in Section 7.1.2, with PK sampling as detailed in the Study Schedule (Attachment 1).

Following termination of the clamp procedure in Period 3 (Day 2), subjects will resume their prestudy insulin treatment regime. Discharge from the CRU will be subject to the same postclamp safety assessments as detailed in Section 7.1.2 and Section 10.4.1. Follow-up will occur 7 to 28 days after the end of Period 3 when safety procedures will be conducted and insulin use and dose will be recorded as detailed in the Study Schedule (Attachment 1).

7.1.4. Blood Glucose Monitoring

Following Period 2 and prior to the Period 3 clamp, MDI study drug doses will be titrated according to subjects' individual needs, as assessed by SMPG results obtained from monitoring at home (subjects will be supplied with the equipment required and trained to conduct SMPG). Site personnel may request additional PG monitoring from subjects and/or assess PG values at other times in order to make dose adjustment decisions.

Subjects should record 4-point SMPG measurements and all insulin doses in the subject diary provided. The mean SMPG measurements for the 5 days prior to Day 1 of Period 3 will be recorded in the electronic case report form (eCRF).

Subjects should be instructed to record 4-point SMPG daily. The 4-point SMPG consists of PG measurements at:

- Pre-morning meal (fasting)
- Pre-midday meal
- Pre-evening meal
- Bedtime (at least 2 hours after the evening meal)

Subjects should also record their 03:00 hours PG readings within 48 hours of any increase in insulin dose titration at telephone or office visits.

During telephone visits, the investigator or site personnel will ask the subject about these PG measurements and recommend dosage titrations based on glucose targets within the TID and BID algorithms in Section 9.4.

Missing values in 4-point SMPG profiles that do not reflect noncompliance with the protocol, in the opinion of the investigator, will not be considered a protocol deviation.

7.2. Discussion of Design and Control

In Part A of this study, a 2-period, randomized, crossover design has been chosen to allow each subject to act as his/her own control. Prestudy U-100, U-200, and/or U-300 insulin will be taken at the same dose and at approximately the same time each day prior to Period 1 and between Periods 1 and 2, and stopped at least 15 hours prior to U-500R bolus administration in Part A in order to reduce variation in insulin carryover (as the bioanalytical methodology is not specific to U-500R).

The use of a euglycemic clamp technique will provide data on the glucodynamic activity of U-500R. This is standard methodology routinely used to safely measure insulin action in clinical studies.

High-dose insulin-treated subjects with T2DM are representative of the population that is prescribed the U-500R formulation. An open-label design is considered appropriate as the PK/PD parameters being investigated in this study are objective endpoints.

Fasting and pre-meal plasma-equivalent glucose target ranges in Period 3 are 71 to 130 mg/dL (3.9 to 7.2 mmol/L), consistent with American Diabetes Association (ADA) standards of care (ADA 2012).

Recommended administration of U-500R bolus doses at least 30 minutes before meals by TID or BID dosing is based on USPI advice and PK/PD study results in obese, healthy subjects, which demonstrate both prandial and basal activity of U-500R (de la Peña et al. 2011).

The 5 to 10 days between Period 2 of Part A and the clamp procedure in Part B is expected to allow for subject recovery between clamps, for titration of doses, and for steady state to be achieved, given the findings of simulation modeling in which either TID or BID dosing steady state was predicted to be achieved by 24 hours (de la Peña et al. 2014).

8. Study Population

8.1. Criteria for Enrollment

Eligibility of subjects for study enrollment will be based on the results of a 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.

Individuals who do not meet the criteria for participation in this study (screen failure) may be re-screened. Individuals may be re-screened 1 time. The interval between screen and re-screen should be at least 2 weeks.

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

8.1.1. Inclusion Criteria

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

[1] males or females with T2DM (World Health Organization [WHO] Classification of Diabetes [Attachment 4]; ADA, 2015).

[1a] female subjects

are women of child-bearing potential who test negative for pregnancy at the time of screening based on a serum pregnancy test, and who agree to use a reliable method of birth control during the study

or

are women not of child-bearing 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 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 (for example oral contraceptives, hormones, gonadotropin releasing hormone, anti-estrogens, selective estrogen receptor modulators, or chemotherapy)

or

2) spontaneous amenorrhea for 6 to 12 months and a follicle-stimulating hormone (FSH) level greater than 40 mIU/mL

[2] are ≥18 to <75 years old at the time of screening

[3] have a BMI of ≥27.0 kg/m^2 at screening

[4] are current users of U-100, U-200, and/or U-300 insulin/analog as basal, premixed, and/or basal/bolus delivered with any injection device (pens and/or syringe/vial but excluding CSII/insulin pump use in the preceding 3 months), taking:

a TDD of ≥150 U

OR

at least one dose >100 U as part of an MDI regimen

AND

TDD ≤3.0 U/kg

[5] Concomitant AHA therapy may include:

- metformin (MET)
- dipeptidyl peptidase-4 inhibitors
- pioglitazone (doses ≤30 mg/day)
- GLP-1 receptor agonists
- sodium-glucose co-transporter-2 (SGLT2) inhibitors, except in combination with GLP-1 receptor agonists (see Exclusion Criterion [31] for excluded AHA therapy).
  Subjects' AHA therapy must have been stable for ≥3 months (except for weekly GLP-1 receptor agonists which must have been stable for ≥4 months).

[6] have HbA1c 7.5% to 11.5%, inclusive, as measured at screening

[7] have venous access sufficient to allow for blood sampling and intravenous administration as per the protocol

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

[9] must be able to safely withhold administration of U-100, U-200, and/or U-300 insulins from ≥15 hours prior to clamp procedures in Periods 1 and 2 as applicable, in the judgment of the investigator

[10] have given written informed consent approved by Lilly and the ethical review board (ERB) governing the site

8.1.2. Exclusion Criteria

Subjects will be excluded from study enrollment if they meet any of the following criteria:

[11] are investigator site personnel directly affiliated with this study and their immediate families. Immediate family is defined as a spouse, parent, child, or sibling, whether biological or legally adopted.

[12] are Lilly employees

[13] are currently enrolled in a clinical trial involving an investigational product or off-label use of a drug or device, or are concurrently enrolled in any other type of medical research judged not to be scientifically or medically compatible with this study

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

[15] are persons who have withdrawn from this study

[16] have T1DM or other types of diabetes mellitus apart from T2DM (WHO Classification of Diabetes [Attachment 4]; ADA 2015)

[17] have known hypersensitivities or allergies to insulin, excipients contained in insulin products, or related compounds

[18] have corrected QT interval (QTc) prolongation >500 ms or have any other abnormality in the 12-lead ECG that, in the opinion of the investigator, increases the risks associated with participating in the study

[19] have an abnormal blood pressure for the study population, as determined by the investigator

[20] have a significant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine (apart from diabetes), hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; of constituting a risk when taking the study medication; or of interfering with the interpretation of data

[21] have known or ongoing psychiatric disorders which in the opinion of the investigator might interfere with subject completion of the study or following of study procedures

[22] regularly use known drugs of abuse and/or show positive findings on urinary drug screening

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

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

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

[26] are women who are pregnant

[27] are women who are lactating

[28] are currently treated with prescription medication (other than those allowed in Inclusion Criteria [4] and [5]), herbal medications, or nutritional supplements that affect PG or insulin sensitivity, or promote weight loss within 4 weeks prior to dosing (Day 1 of Period 1). Initiation of new over-the-counter medication within 7 days prior to dosing, or prescription medications or herbal medication within 14 days prior to dosing may be permitted following discussion with the Lilly clinical pharmacologist. Occasional intake of acetaminophen or vitamin/mineral supplements will be allowed. Ongoing medications (such as hormonal contraceptives, thyroid replacement therapy, anti-hypertensives, low-dose aspirin, or cholesterol-lowering agents) may be permitted at unchanged doses (Section 9.7).

[29] have used systemic glucocorticoids within 4 weeks prior to screening

[30] have used U-500R within 3 months prior to screening

[31] have used rosiglitazone, pramlintide, once-weekly or BID exenatide, or other injectable or oral AHA not listed in Inclusion Criterion [5] in the 3 months prior to screening; or are taking oral antidiabetic medications at doses exceeding the respective product labels; or have a contraindication to current oral antidiabetic medication usage per the respective product labels (for example, MET: serum creatinine ≥1.4 mg/dL in women or ≥1.5 mg/dL in men).

[32] have donated blood of more than 500 mL within the last month

[33] have an average weekly alcohol intake that exceeds 21 units per week (males up to age 65) and 14 units per week (males over 65 and females), or are unwilling to stop alcohol consumption for 24 hours prior to each clamp procedure and while resident in 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)

[34] are smokers who smoke >10 cigarettes or equivalent per day and/or are not able or willing to refrain from smoking, using electronic cigarettes or the using nicotine replacement therapy (for example, nicotine gum or patches) while resident in the CRU

[35] are excessive consumers of xanthines (more than 5 cups of tea, coffee, cola, or hot chocolate per day)

[36] have had a blood transfusion or severe blood loss within 3 months prior to screening or have known hemoglobinopathy, hemolytic anemia, or sickle cell anemia that may affect reliability of HbA1c measurements

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

8.1.3. Rationale for Exclusion of Certain Study Candidates

Exclusion Criteria 11 and 12 prevent conflict of interest in study participants. Criteria 13 through 35 and 37 exclude medical conditions, medication intolerance, and concomitant

medication use that may constitute a risk for the subject and/or may confound the assessment of study endpoints. Criterion 36 addresses issues that could have an impact on HbA1c values that are not related to glycemic control.

8.2. Discontinuation

The reason for and date of discontinuation will be collected for all subjects. All enrolled subjects who discontinue, regardless of whether they received investigational product, will have procedures performed as shown in the Study Schedule (Attachment 1).

8.2.1. Discontinuation of Subjects

8.2.1.1. Subjects Inadvertently Enrolled

The criteria for enrollment must be followed explicitly. If the investigator site identifies a subject who did not meet enrollment criteria and who was inadvertently enrolled, the sponsor must be notified. If the sponsor identifies a subject who did not meet enrollment criteria and who was inadvertently enrolled, the investigator site will be notified. A discussion must occur between the Lilly clinical pharmacologist or clinical research physician (CRP) and the investigator to determine whether the subject may continue in the study, with or without investigational product. Inadvertently enrolled subjects may be maintained in the study and on investigational product when the investigator and Lilly clinical pharmacologist or CRP agree it is medically appropriate for that subject to continue. The subject may not continue in the study with or without investigational product if the Lilly clinical pharmacologist or CRP does not agree with the investigator's determination it is medically appropriate for the subject to continue. The investigator must obtain documented approval from the Lilly clinical pharmacologist or CRP to allow the inadvertently enrolled subject to continue in the study with or without investigational product.

8.2.1.2. Discontinuations from Investigational Product and from the Study

Subjects will be discontinued from the investigational product and from the study in the following circumstances:

- Enrollment in any other clinical trial involving an investigational product or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study.
- If the subject's body weight varies by more than 5 kg between the clamp procedures in Periods 1 and 2.
- Investigator Decision.
    - The investigator or attending physician decides that the subject should be discontinued from the study.
    - If the subject, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of the study indication, discontinuation from the study occurs prior to introduction of the other agent.

- If the investigator determines that the subject is significantly noncompliant with study drug, glucose monitoring, completion of the subject diary, or other study procedures, and should be discontinued.

- Subject Decision
  - the subject requests to be discontinued from the study
- Sponsor Decision
  - Lilly stops the study or stops the subject's participation in the study for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP).
- Adverse Event
  - If a clinically significant event (CSE) occurs, the investigational product is to be discontinued and appropriate measures taken. Lilly or its designee should be alerted immediately. A CSE will be defined as a moderate to severe adverse event (AE), abnormal clinical sign, or clinical laboratory finding that may pose a risk to the well-being of the subject. Refer to Safety Evaluations Section (Section 10.4).
  - A clinically significant systemic hypersensitivity reaction occurs follow ing administration of the investigational product (for example, drug-related symptomatic bronchospasm, allergy-related edema/angioedema, or hypotension), that requires parenteral medication, does not respond to symptomatic medication, or results in clinical sequelae or an anaphylactic reaction.

Following the investigator's determination that CSE criteria have been met and the investigator's judgment of relatedness to the investigational product is documented, a decision will be made between the investigator and Lilly or its designee regarding subject discontinuation.

The nature of any conditions, clinical signs or symptoms, or abnormal laboratory values present at the time of discontinuation and any applicable follow-up procedures will be documented.

Refer to the Study Schedule (Attachment 1) for data collected at the time of discontinuation and follow-up.

8.2.1.3. 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 study site. Site 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 site.

8.2.2. Discontinuation of Study Sites

Study site participation may be discontinued if Lilly, the investigator, or the ERB of the study site judges it necessary for any reason consistent with applicable laws, regulations, and GCP.

8.2.3. 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.

9. Treatment

9.1. Treatment Materials and Supplies

This study involves a comparison of a 100-U bolus of U-500R administered by single SC injection versus administration via CSII and evaluation of U-500R administered SC TID and BID. Table IBHG.9.1 shows the treatment regimens.

Table IBHG.9.1. Investigational Product Regimens

	Regimen	Dose	Administration route	Time of administration
Part A	U-500R SC bolus	100-U	Single SC injection	Prior to initiation of euglycemic clamp
	U-500R basal infusion plus U-500R CSII bolus	4.25 U/h basal and 100-U bolus	CSII	12 hour infusion of 4.25 U/h U-500R prior to 100-U bolus dose and initiation of euglycemic clamp (with continued 4.25-U/h U-500R infusion)
Part B	U-500R TID	Per titration algorithm (TID, injectable solution)	Multiple daily SC injection	Recommended 07:00, 12:00, and 18:00 hours, approximately 30 minutes prior to morning meal, midday meal, and evening meal, respectively[a]
	U-500R BID	Per titration algorithm (BID, injectable solution)	Multiple daily SC injection	Recommended 07:00 and 18:00 hours, approximately 30 minutes prior to morning meal and evening meal, respectively[a]

Abbreviations: BID = twice daily; CSII = continuous subcutaneous insulin infusion; SC = subcutaneous; TID = thrice daily; U-500R = human regular U-500 insulin.

[a] If a meal is skipped, insulin dose should be reduced by 50%.

9.2. Treatment Administration

The investigator or designee is responsible for:

- explaining the correct use of the investigational product to the subjects and site personnel,
- verifying that instructions are followed properly,

- maintaining accurate records of investigational product dispensing and collection,
- and returning all unused medication to Lilly or its designee at the end of the study.

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

Subjects will be instructed to contact the investigator as soon as possible if he or she has a complaint or problem with the investigational product or drug delivery system so that the situation can be assessed.

All clinical trial material provided to the investigator will be stored in a secure place and allocated and dispensed by appropriately trained persons. The allocation and dispensing of the investigational products will be fully documented and verified by a second person. Detailed records of the amounts of the investigational product received, dispensed, and remaining at the end of the study will be maintained. Not in-use or unopened Humulin R U-500 vials should be stored in a refrigerator, (2°C to 8°C [36° F to 46°F]), but not in the freezer. Once opened, the U-500R vial can be kept unrefrigerated as long as it is kept as cool as possible (below 30°C [86°F]) and away from heat and light. In-use vials must be used within 40 days, even if they still contain U-500R (Humulin R U-500 Package Insert, 2014). Clinical trial materials will be labeled according to the respective country's regulatory requirements.

During periods of prestudy U-100, U-200, and/or U-300 insulin treatment, subjects will self-administer their insulin at home, at approximately the same time each day. The last dose prior to assessment should be taken at approximately the same time in each period and will be at least 15 hours before the U-500R bolus dose and clamp procedure in Part A. The insulin TDD should remain approximately the same throughout Part A of the study (except while subjects are resident in the CRU when it will not be taken).

The insulin preparation will be allowed to come to approximately room temperature prior to administration. The qualified site personnel will be instructed on proper preparation and administration techniques for all insulin used in this study. All insulin will be administered by an appropriately qualified member of the CRU staff while the subject is resident in the CRU.

All SC insulin injections will be given by means of a conventional U-100 insulin syringe (6 mm, 31-gauge needle; 0.5 or 1.0 mL). The same brand and model of syringe will be used throughout the study. The needle will be applied at approximately 90 degrees into a raised skin fold to ensure consistent SC administration. The injection site will be alternated between the 4 quadrants of the abdominal wall, approximately 10 cm from the umbilicus.

In Part A, 100 U of U-500R will be administered by single SC injection or CSII according to the randomization schedule.

For CSII administration, U-500R will be administered via a U-500 OmniPod (currently not available commercially), which will be fitted by qualified staff upon subjects' admission to the CRU. The device will be programmed by CRU staff to administer a 12-hour 4.25-U/h infusion

of U-500R, prior to the 100-U bolus and to continue the 4.25-U/h infusion throughout the clamp procedure. The insulin-on-board function on the U-500 OmniPod will be set at 6 hours. The Pod will be filled from the 20-mL vial of U-500R using only the syringe and needle provided with the U-500 OmniPod by the vendor.

The unit markings on a U-100 insulin syringe do not accurately reflect the unit dose of U-500R because U-500R is 5-times more concentrated than U-100 insulin, and thus only 1/5 the volume is needed to give an equivalent dose to U-100 insulin.

In Part A, for subjects randomized to SC administration, a qualified member of CRU staff will administer 100 U of U-500R, equivalent to 20 unit markings on a 0.5-mL U-100 syringe.

In Part B, CRU staff should instruct subjects on the dose conversion of calculated U-500R to the U-100 syringe unit markings (detailed instruction for which will be provided separately to the site).

All U-500R doses ≤250 U should be administered using 0.5-mL U-100 insulin syringes. All U-500R doses >250 U should be administered using 1.0-mL U-100 insulin syringes.

For example:

- 155 U of U-500R have a volume equivalent to 31 U markings on a 0.5 mL U-100 syringe (155 divided by 5=31).
- 100 U of U-500R have a volume equivalent to 20 U markings on a 0.5 mL U-100 syringe (100 divided by 5=20).
- 300 U of U-500R have a volume equivalent to 60 U markings on a 1.0 mL U-100 syringe (300 divided by 5=60).

In Part B, U-500R doses determined as described in Section 9.4 will be administered either TID or BID according to the randomization schedule. The doses will be administered at approximately the same times on each day. The actual time of all dose administrations will be recorded in the subject's eCRF.

9.3. Rationale for Selection of Dose

A dose of 100 U of U-500R was chosen for Part A of this study as this is within the range commonly encountered in high-dose insulin-treated patients whose T2DM is managed with U-500R either by MDI or off-label via CSII (Lane et al. 2009; de la Peña et al. 2011; Reutrakul et al. 2012; Cochran et al. 2014; and Lane et al. 2013).

Study dosing and conversion and titration regimens for the TID and BID treatment groups in Part B, both guided by pre-Period 2 U-100, U-200, and/or U-300 insulin TDD (based on the highest TDD recorded in the 3 days prior to Period 2 which should be approximately unchanged from pre-Period 1) and baseline HbA1c (screening), and subsequent glucose monitoring, respectively, are based on literature expert reviews (Lane et al. 2009; Segal et al. 2010; Reutrakul, et al. 2012) and on the results from simulations performed using a systems physiology model of diabetes (in Physiolab®; de la Peña et al. 2014) based on data from previous PK/PD studies of

U-500R and U-100 human regular insulin (de la Peña et al. 2011; Studies F3Z-LC-IMAC and H7U-MC-IDBM).

9.4. Dose Conversion from Prestudy Insulin in Part B

Subjects will record their insulin doses throughout the study. Baseline U-100, U-200, and/or U-300 insulin TDD will be defined as the highest TDD recorded in the 3 days prior to Period 2. All U-100, U-200, and/or U-300 insulin therapy will be discontinued upon initiation of study drug, but permitted prestudy AHA therapy should continue to be taken at prestudy doses, except on clamp assessment days. For all subjects with HbA1c >8.0% at screening, initial U-500R TDD will be the same (in units) as U-100, U-200, and/or U-300 insulin TDD at screening.

For all subjects with baseline HbA1c ≤8.0% at screening, initial U-500R TDD dose will be reduced by 20% from the U-100, U-200, and/or U-300 insulin TDD at screening.

Initial TID dosing will use a 40:30:30 proportion of calculated U-500R TDD for the respective pre-meal doses at 07:00 hours, 12:00 hours, and 18:00 hours, approximately 30 minutes prior to the morning meal, midday meal, and evening meal, respectively.

Initial BID dosing will use a 60:40 proportion of calculated U-500R TDD for the respective pre-meal doses at 07:00 hours and 18:00 hours, approximately 30 minutes prior to the morning meal and evening meal, respectively.

During dose conversion, bolus doses will be rounded down to the nearest 5 U as was done in Study B5K-US-IBHC (Hood et al. 2015). If subjects are using 0.5-mL U-100 insulin syringes, this will be to the closest unit marking (0.01 mL) on the syringe; for those using 1.0-mL U-100 insulin syringes, this will be half way between unit markings (0.02 mL).

Subjects will record SMPG throughout Period 3. During telephone visits (conducted 2 days after the end of Period 2 at a minimum) the investigator or designee will titrate subjects' U-500R dose for each meal according to the algorithms presented in Table IBHG.9.2.

During dose titration (as opposed to initial dosage conversion), conventional rounding to the nearest 5 U will be applied, so that, for instance, values between 120.1 and 122.4 would be rounded down to 120 U while values between 122.5 and 124.9 would be rounded up to 125 U.

Subjects should perform a 03:00 hours SMPG within 48 hours of any dose increase and should telephone the CRU the next morning if their 03:00 hours SMPG value is ≤70 mg/dL (≤3.9 mmol/L). The investigator may deviate from the algorithms, if deemed necessary, for the safety or well-being of the subject (for example due to illness, unusual SMPG patterns, or other safety concerns). Deviations from the algorithm should be discussed with Lilly, in a dvance if possible without compromising subject safety.

In the event of a subject reporting a severe hypoglycemic episode or any SMPG value is <50 mg/dL (<2.8 mmol/L), the investigator will make appropriate insulin adjustment per his/her judgment (this may be a 15% reduction in the appropriate U-500R mealtime dosage).

If a meal is skipped during the treatment period, the U-500R dose should be reduced by 50%.

Table IBHG.9.2. TID and BID Dosing Algorithms for U-500R Using Plasma-Equivalent Glucose Values

Algorithm	Insulin Dose to Adjust	Plasma-Equivalent Glucose Value	Value (mg/dL)	Action
TID Initial Dose Proportion 40:30:30	Pre-morning meal	Median pre-midday meal	≤70 71-130 131-180 181-220 >220	Reduce dose by 10% No change in dose Increase dose by 5% Increase dose by 10% Increase dose by 15%
	Pre-midday meal	Median pre-evening meal SMPG	≤70 71-130 131-180 181-220 >220	Reduce dose by 10% No change in dose Increase dose by 5% Increase dose by 10% Increase dose by 15%
	Pre-evening meal	Median pre-morning meal, median bedtime, or 03:00 hours SMPG	≤70	Reduce dose by 10%
		Median pre-morning meal SMPG	71-130 131-180 181-220 >220	No change in dose Increase dose by 5% Increase dose by 10% Increase dose by 15%
BID Initial Dose Proportion 60:40	Pre-morning meal	Median pre-evening meal or median pre-midday meal SMPG	≤70	Reduce dose by 10%
		Median pre-evening meal SMPG	71-130 131-180 181-220 >220	No change in dose Increase dose by 5% Increase dose by 10% Increase dose by 15%
	Pre-evening meal	Median pre-morning meal, median bedtime, or 03:00 hours SMPG	≤70	Reduce dose by 10%
		Median pre-morning meal SMPG	71-130 131-180 181-220 >220	No change in dose Increase dose by 5% Increase dose by 10% Increase dose by 15%

Abbreviations: BID = twice daily; SMPG = self-monitored plasma glucose; TID = thrice daily.

9.4.1. TID Treatment Group Titrations

Subjects in the TID treatment group will have their insulin doses adjusted at a telephone visit 2 to 3 days after the end of Period 2 (at a minimum, additional telephone or office visits may be added for safety as necessary) by the investigator or site designee according to the TID titration algorithm (Table IBHG.9.2).

The U-500R doses will be changed according to corresponding median plasma-equivalent glucose readings from the most recent 3 values at respective times (also see examples below) prior to the telephone or office visit or for any add-on telephone calls or office visits. If even

1 SMPG reading for a given time point is ≤70 mg/dL (≤3.9 mmol/L), no dosage increase will be applied to the corresponding dose. These adjustments will be applied only to the 2 of the 3 doses (pre-morning meal, pre-midday meal, pre-evening meal) that most need adjustment (that is, the ones with the most out-of-range corresponding median plasma-equivalent glucose readings) at each study visit/interaction.

For subjects receiving U-500R TID, median pre-morning meal and bedtime SMPG values and 03:00 hours SMPG values (if performed) will inform adjustment of the pre-evening meal U-500R insulin dose. When these 3 sets of values provide conflicting guidance, the investigator should base the dose adjustment on the lowest of these results, and prioritize adjustments for hypoglycemic readings ≤70 mg/dL (≤3.9 mmol/L) over dosage increases.

In all instances, the 10% dosage reduction for values ≤70 mg/dL (≤3.9 mmol/L) and negation of respective dosage increases for single pre-meal or bedtime hypoglycemic readings should be prioritized over other titration options.

Example 1

A subject assigned to the TID treatment group is taking 300 U TDD with a pre-morning meal dose of 120 U, pre-midday meal dose of 90 U and a pre-evening meal dose of 90 U.

Over the last 3 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3
SMPG pre-morning meal, mg/dL (mmol/L)	102 (5.7)	110 (6.1)	135 (7.5)
SMPG pre-midday meal, mg/dL (mmol/L)	163 (9.1)	180 (10.0)	200 (11.1)
SMPG pre-evening meal, mg/dL (mmol/L)	240 (13.3)	221 (12.3)	238 (13.2)
SMPG at bedtime, mg/dL (mmol/L)	130 (7.2)	160 (8.9)	125 (6.9)
SMPG at 03:00 hours, mg/dL (mmol/L)			92 (5.1)

Abbreviation: SMPG = self-monitored plasma glucose.

The 2 doses that most need adjustment are pre-midday meal and pre-morning meal because the median glucose values at pre-evening meal and pre-midday meal are the most outside of the target range. The subject's median pre-evening meal SMPG is 238 mg/dL (13.2 mmol/L); therefore, the pre-midday meal dose should be increased by 15% to 103.5 U, rounded up to 105 U. The subject's median pre-midday meal SMPG is 180 mg/dL (10.0 mmol/L); therefore, the pre-morning meal dose should be increased by 5% to 126 U, rounded down to 125 U.

Example 2:

A subject assigned to the TID treatment group is taking 210 U TDD with a pre-morning meal dose of 90 U, pre-midday meal dose of 60 U, and pre-evening meal dose of 60 U.

Over the last 3 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3
SMPG pre-morning meal, mg/dL (mmol/L)	160 (8.9)	145 (8.1)	110 (6.1)
SMPG pre-midday meal, mg/dL (mmol/L)	181 (10.1)	185 (10.3)	120 (6.7)
SMPG pre-evening meal, mg/dL (mmol/L)	140 (7.8)	120 (6.7)	170 (9.4)
SMPG at bedtime, mg/dL (mmol/L)	68 (3.8)	120 (6.7)	140 (7.8)
SMPG at 03:00 hours, mg/dL (mmol/L)		60 (3.3)	

Abbreviation: SMPG = self-monitored plasma glucose.

The 2 doses that appear to most need adjustment are pre-morning meal and pre-evening meal because the median glucose values at pre-midday meal and pre-morning meal are the most outside of the target range (181 and 145 mg/dL (10.1 and 8.1 mmol/L), respectively). However, due to the 03:00 hours SMPG being ≤70 mg/dL (≤3.9 mmol/L), the first prioritized dose change would be the subject's pre-evening meal dose which should be reduced by 10% to 54 U, rounded up to 55 U. After that first dose adjustment, the next adjustment most needed would be a 10% increase in the subject's pre-morning meal dose to 99 U, rounded up to 100 U on the basis of the pre-midday median glucose value of 181 mg/dL (10.1 mmol/L). Since only 2 of 3 TID doses can be adjusted, no change would be applied to the pre-midday meal dose.

Example 3:

A subject assigned to the TID treatment group is taking 300 U TDD with a pre-morning meal dose of 120 U, pre-midday meal dose of 90 U, and pre-evening meal dose of 90 U.

Over the last 4 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3	Day 4
SMPG pre-morning meal, mg/dL (mmol/L)	66 (3.7)	148 (8.2)		160 (8.9)
SMPG pre-midday meal, mg/dL (mmol/L)	120 (6.7)	142 (7.9)	180 (10.0)	
SMPG pre-evening meal, mg/dL (mmol/L)	68 (3.8)	64 (3.6)		135 (7.5)
SMPG at bedtime, mg/dL (mmol/L)	102 (5.7)	135 (7.5)		165 (9.2)
SMPG at 03:00 hours, mg/dL (mmol/L)		100 (5.6)		

Abbreviation: SMPG = self-monitored plasma glucose.

The investigator should consider changing only the 2 doses that most need adjustment. In this instance, the 2 doses that appear to most need adjustment are pre-evening meal and pre-morning meal because the median glucose values at pre-morning meal and pre-evening meal are the most outside of the target range. The median pre-morning meal SMPG (148 mg/dL [8.2 mmol/L]) is within the range of 131 to 180 mg/dL (7.3 to 10.0 mmol/L) calling for a 5% dose increase of pre-evening meal dosage. However, the unexplained hypoglycemia (66 mg/dL [3.7 mmol/L]) takes precedence and accordingly, there should be no upward evening meal dosage adjustment. The median of the last 3 readings for pre-evening meal SMPG (68 mg/dL [3.8 mmol/L]) is ≤70 mg/dL (≤3.9 mmol/L); therefore, the investigator would reduce the pre-midday meal dose by 10% to 81 U, which is rounded to 80 U, the nearest 5 U.

9.4.2. BID Treatment Group Titrations

Subjects in the BID treatment group will have their insulin dosages adjusted at each study visit (office and telephone or for add-on visits) by the investigator or site designee according to the BID titration algorithm (Table IBHG.9.2).

Human regular U-500 insulin doses will be changed according to corresponding median plasma-equivalent glucose readings from the most recent 3 values at respective times (also see examples below) prior to the scheduled telephone or office visit or for any add-on visits. If even 1 of the SMPG readings for a given time point is ≤70 mg/dL (≤3.9 mmol/L), no dosage increase will be applied to the corresponding dose. These adjustments will be applied to both doses as indicated (pre-morning meal and pre-evening meal) at each study visit/interaction.

For subjects receiving U-500R BID, median pre-morning and bedtime SMPG values and 03:00 hours SMPG values (if performed) will inform adjustment of the pre-evening meal U-500R insulin dose, and both median pre-evening meal and pre-midday meal SMPG values will inform adjustment of the pre-morning meal U-500R insulin dose. When these sets of values provide conflicting guidance, investigators should base the dose adjustment on the lowest of these results, and prioritize adjustments for hypoglycemic readings ≤70 mg/dL (≤3.9 mmol/L) over dosage increases.

In all instances, the 10% dosage reduction for median pre-meal/bedtime values or single 03:00 hours glucose values ≤70 mg/dL (≤3.9 mmol/L) and negation of respective dosage increases for single pre-meal or bedtime hypoglycemic readings should be prioritized over other titration options.

Example 1:

A subject assigned to the BID treatment group is taking 300 U TDD, with a pre-morning meal dose of 180 U and a pre-evening meal dose of 120 U.

Over the last 4 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3	Day 4
SMPG pre-morning meal, mg/dL (mmol/L)	180 (10.0)	160 (8.9)	200 (11.1)	
SMPG pre-midday meal, mg/dL (mmol/L)		120 (6.7)	170 (9.4)	132 (7.3)
SMPG pre-evening meal, mg/dL (mmol/L)	200 (11.1)		225 (12.5)	260 (14.4)
SMPG at bedtime, mg/dL (mmol/L)	142 (7.9)		128 (7.1)	110 (6.1)
SMPG at 03:00 hours, mg/dL (mmol/L)	101 (5.6)			

Abbreviation: SMPG = self-monitored plasma glucose

The median pre-evening meal SMPG value is 225 mg/dL (12.5 mmol/L); therefore, the subject's pre-morning meal dose should be increased by 15% to 207 U, rounded down to 205 U. The median pre-morning meal SMPG value is 180 mg/dL (10.0 mmol/L); therefore, the subject's pre-evening meal dose should be increased by 5% to 126 U, rounded down to 125 U.

Example 2:

A subject assigned to the BID treatment group is taking 250 U TDD, with a pre-morning meal dose of 150 U and a pre-evening meal dose of 100 U.

Over the last 4 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3	Day 4
SMPG pre-morning meal, mg/dL (mmol/L)		138 (7.7)	130 (7.2)	110 (6.1)
SMPG pre-midday meal, mg/dL (mmol/L)	80 (4.4)	71 (3.9)	58 (3.2)	
SMPG pre-evening meal, mg/dL (mmol/L)	160 (8.9)		200 (11.1)	182 (10.1)
SMPG at bedtime, mg/dL (mmol/L)	140 (7.8)		132 (7.3)	110 (6.1)
SMPG at 03:00 hours, mg/dL (mmol/L)		142 (7.9)		

Abbreviation: SMPG = self-monitored plasma glucose.

The median pre-evening meal SMPG is 182 mg/dL (10.1 mmol/L); thus, the subject's pre-morning meal dose would normally be increased by 10%. However, there is a single pre-midday meal hypoglycemic reading (58 mg/dL [3.2 mmol/L]); therefore, the pre-morning meal dose should not be increased. The median pre-morning meal SMPG value is 130 mg/dL (7.2 mmol/L); therefore, the subject's pre-evening meal dose should not change.

Example 3:

A subject assigned to the BID treatment group is taking 275 U TDD with a pre-morning meal dose of 165 U and a pre-evening meal dose of 110 U.

Over the last 3 days, the subject's SMPG values were:

Date	Day 1	Day 2	Day 3
SMPG pre-morning meal, mg/dL (mmol/L)	66 (3.7)	141 (7.8)	160 (8.9)
SMPG pre-midday meal, mg/dL (mmol/L)	131 (7.3)	128 (7.1)	105 (5.8)
SMPG pre-evening meal, mg/dL (mmol/L)	66 (3.7)	70 (3.9)	110 (6.1)
SMPG at bedtime, mg/dL (mmol/L)	100 (5.6)	112 (6.2)	108 (6.0)
SMPG at 03:00 hours, mg/dL (mmol/L)	70 (3.9)		

Abbreviation: SMPG = self-monitored plasma glucose.

The median pre-evening meal SMPG is 70 mg/dL (3.9 mmol/L); therefore, the pre-morning meal dose should be reduced by 10% to 148.5 U, rounded up to 150 U. The median pre-morning meal SMPG is 141 mg/dL (7.8 mmol/L), but the subject's pre-evening meal dose should not be titrated upward due to the 03:00 hours value being ≤70 mg/dL (≤3.9 mmol/L) (70 mg/dL [3.9 mmol/L]) which also necessitates a 10% reduction, in the pre-evening meal dose to 99 U, rounded up to 100 U.

9.5. Specific Restrictions/Requirements

Prior to beginning the study, the subjects will complete informed consent and baseline tests.

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

Meals/Diet – For all treatment periods, subjects will be fasted from approximately 12 hours prior to clamp initiation until after the clamp procedure is completed. Water can be consumed freely during this period.

When at home, subjects will be encouraged to maintain their body weight and consume their normal diets.

Alcohol – No alcohol will be allowed from at least 24 hours prior to clamp initiation and while resident in the CRU.

Smoking – Subjects will refrain from smoking, using electronic cigarettes, or using nicotine replacement therapy while resident in the CRU.

Exercise – Subjects will be encouraged to maintain their regular exercise; however, they should not undertake vigorous or prolonged exercise within 48 hours prior to each clamp day.

After dosing, and during the clamp procedure, subjects should remain recumbent or sitting in the CRU. Movement will be restricted to retain integrity of connections to infusion and study procedures.

Authorization to leave the CRU must be given by a physician.

9.6. Blinding

This is an open-label study.

9.7. Concomitant Therapy

Subjects on stable concomitant medication at the time of study entry should continue their regular, unchanged dose throughout the study; for example, hormonal contraceptives, thyroid replacement therapy, anti-hypertensives, low-dose aspirin, and cholesterol lowering agents. Prescription medications (other than those allowed in Inclusion Criteria [4] and [5]), herbal medications, or nutritional supplements that affect PG or insulin sensitivity, or promote weight loss will not be allowed within 4 weeks prior to dosing. Initiation of new over-the-counter medication within 7 days prior to dosing, or prescription medication or herbal medication within 14 days prior to dosing may be permitted following discussion with the Lilly clinical pharmacologist. Vitamin/mineral supplements and/or occasional use of acetaminophen will be allowed.

Subjects may continue to take their stable prestudy AHA dose (MET, dipeptidyl peptidase-4 inhibitors, pioglitazone [doses ≤30 mg/day], GLP-1 receptor agonists, and/or SGLT2 inhibitors [not in combination with GLP-1 receptor agonists]), unchanged, throughout the study, except on clamp assessment days.

Rosiglitazone, pramlintide, once weekly or BID exenatide, other injectable or oral AHAs not listed in Inclusion Criterion [5], or permitted AHAs taken at doses other than that prescribed by the product label will not be allowed.

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

10. Sample Collection and Safety Data Collection

Attachment 1 lists the schedule for sample collections in this study.

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

Attachment 3 summarizes the blood volumes for all blood sampling during the study.

10.1. Laboratory Samples

Blood samples will be collected to determine whether subjects meet inclusion/exclusion criteria and to monitor subject health.

Investigators must document their review of each laboratory safety report.

Samples collected for specified laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Tests are run and confirmed promptly whenever scientifically appropriate. When scientific circumstances warrant, however, it is acceptable to retain samples to batch the tests run, or to retain the samples until the end of the study to confirm that the results are valid. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

10.2. Samples for Pharmacokinetic and Pharmacodynamic Evaluations

10.2.1. Pharmacokinetic Samples

At the visits and times specified in the Study Schedule, venous blood samples of approximately 3.5 mL and 2.5 mL each will be collected to determine the serum IRI and C-peptide concentrations, respectively. Instructions for the collection and handling of blood samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sampling will be recorded.

10.2.2. Bioanalysis

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

Concentrations of IRI will be assayed using a validated enzyme linked immunosorbent assay (ELISA) method for the quantitative determination of IRI. This method measures endogenous insulin, as well as exogenous biosynthetic insulin, after a pretreatment of the sample with polyethylene glycol to remove anti-insulin antibodies. C-peptide measurements will be used to correct for endogenous insulin production.

Bioanalytical samples collected to measure investigational product concentrations will be retained for a maximum of 1 year following last subject visit for the study.

10.2.3. Pharmacodynamic Evaluations (Glucose Clamp Procedure)

The aim of the euglycemic glucose clamp is to maintain euglycemia through glucose infusion after the administration of a dose of insulin. During the glucose clamp, the GIR will be adjusted to maintain a predetermined target blood glucose (BG) concentration for the individual subject. The BG concentration will be maintained within approximately ±5% of the predose target value, which will be defined as 120 mg/dL (6.7 mmol/L).

Thus BG concentrations will be kept constant while GIR varies. The varying GIR will reflect the PD activity of insulin.

Subjects will participate in euglycemic clamps on 3 separate visits. Clamp procedures in Part A will last up to 24 hours after dosing, and clamp procedures in Part B will last 24 hours. The GIRs required to maintain euglycemia and BG concentrations will be documented throughout the procedure. If a clamp cannot be completed due to logistical reasons (for example, lack of venous access), the subject may return on 1 additional occasion for the clamp assessment.

10.3. Samples for Biomarker Research

There is growing evidence that genetic variation may impact a patient's response to therapy. Variable response to therapy may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion, the mechanism of action of the drug, the disease etiology and/or the molecular subtype of the disease being treated. Therefore, where local regulations and ERBs allow, a blood sample will be collected for pharmacogenetic analysis.

In the event of an unexpected AE or the observation of unusual response, the pharmacogenetic samples may be genotyped and analysis may be performed to evaluate a genetic association with response to the investigational product. These investigations may be limited to a focused candidate gene study or, if appropriate, genome wide analysis may be performed to identify regions of the genome associated with the variability observed in drug response. The pharmacogenetic samples will only be used for investigations related to disease and drug or class of drugs under study in the context of this clinical program. They will not be used for broad exploratory unspecified disease or population genetic analysis.

The samples will be coded with the subject number and stored for up to a maximum 15 years after the last subject visit for the study at a facility selected by the sponsor. The samples and any data generated from them can only be linked back to the subject by investigator site personnel. The duration allows the sponsor to respond to regulatory requests related to the investigational product.

Samples will be destroyed according to a process consistent with local regulation.

10.4. Safety Evaluations

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. Planned safety assessments and measures are detailed in Section 10.4.5, but additional assessments and safety tests may be performed at the investigator's discretion.

The investigator remains responsible for following, through an appropriate health care option, AEs that are serious, considered related to study treatment or the study, or that caused the subject to discontinue before completing the study. The subject should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

In addition to records of observations made at specific times, unexpected signs and symptoms and concomitant medications will be recorded in the clinical trial records throughout the study.

10.4.1. Safety Measures

At the end of the glucose clamp, the subject will receive a meal and undergo medical assessment before discharge. If glucose is still being infused at the time of clamp discontinuation, the subject will be monitored for safety. This may consist of hourly PG determinations and monitoring for continuing signs or symptoms of hypoglycemia. The subject will be observed until stable according to investigator judgment. Per investigator judgment, appropriate measures for symptomatic hypoglycemia and/or PG ≤70 mg/dL (≤3.9 mmol/L) will be applied. These measures may include oral glucose or glucose-containing food or drink, glucagon, or parenteral glucose, with follow-up testing, also according to investigator judgment.

10.4.2. Adverse Events

Lilly has standards for reporting AEs that are to be followed regardless of applicable regulatory requirements that may be less stringent.

Study site personnel will record the occurrence and nature of each subject's preexisting conditions, including clinically significant signs and symptoms of the disease under treatment in the study.

Cases of pregnancy that occur during maternal or paternal exposures to the investigational product and/or drug delivery system should be reported. Data on fetal outcome and breast-feeding are collected for regulatory reporting and drug safety evaluation.

After the informed consent form (ICF) is signed, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs. All AEs related to protocol procedures are reported to Lilly or designee.

Any clinically significant findings from ECGs, labs, vital sign measurements, or other procedures, should be reported as an AE to Lilly or its designee.

In addition, all AEs occurring after the subject receives the first dose of the investigational product must be reported to Lilly or its designee via eCRF.

Investigators will be instructed to report to Lilly or its designee their assessment of the potential relatedness of each AE to protocol procedure, studied disease state, investigational product, and/or drug delivery system via eCRF.

The investigator will decide whether he or she interprets the observed AEs as either related to disease, to the study medication, study procedure, drug delivery system, or other concomitant treatment or pathologies. To assess the relationship of the AE to the investigational product, the following terminologies are defined:

- **Related**: a direct cause and effect relationship between the study treatment and the AE is likely.
- **Possibly related**: a cause and effect relationship between the study treatment and the AE has not been demonstrated at this time and is not probable, but is also not impossible.
- **Unrelated**: without question, the AE is definitely not associated with the study treatment.

As per Lilly's standard operating procedures, all "related" and "possibly related" AEs and SAEs will be defined as related to the investigational product.

If a subject's dosage is reduced or treatment is discontinued as a result of an AE, study site personnel must clearly report to Lilly or its designee via eCRF the circumstances and data leading to any such dosage reduction or discontinuation of treatment.

10.4.3. Serious Adverse Events

All episodes of hypoglycemia that are determined by the investigator to constitute severe hypoglycemia according to the definition in Section 10.4.4 should be reported as SAEs.

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

- 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
- considered significant by the investigator for any other reason

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

When a condition related to the insulin pump necessitates medical or surgical intervention to preclude either permanent impairment of a body function or permanent damage to a body structure, the serious outcome of "required intervention" will be assigned.

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

Study site personnel must alert Lilly 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.

Serious adverse event collection begins after the subject has signed the ICF and has received investigational product. If a subject experiences an SAE after signing informed consent, but prior to receiving investigational product, the event will NOT be reported as serious unless the investigator feels the event may have been caused by a protocol procedure.

Serious adverse events occurring up to and including the subject's last study visit will be collected, regardless of the investigator's opinion of causation, in the clinical data collection database and the pharmacovigilance system at the sponsor.

The investigator does not need to actively monitor subjects for AEs once the trial has ended unless specified in the protocol. However, if an investigator becomes aware of SAEs occurring to a subject after the subject's participation in the trial has ended, the investigator should report them to the sponsor, regardless of the investigator's opinion of causation, and the SAEs will be entered in the pharmacovigilance system at the sponsor.

Information on SAEs expected in the study population independent of drug exposure and that will be assessed by the sponsor in aggregate periodically during the course of the trial may be found in the USPI.

10.4.3.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the USPI and that the investigator identifies as related to investigational product or procedure. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. 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.

10.4.4. Hypoglycemia

Subjects will be encouraged to perform SMPG whenever hypoglycemia may be suspected, either by symptoms experienced or perceived increased risk as related to dietary intake, physical activity, or inadvertent or atypical insulin dosing. All subjects will be instructed to treat an

SMPG ≤70 mg/dL (≤3.9 mmol/L) as hypoglycemia. All subjects will be advised to contact the study site if they experience severe hypoglycemia or SMPG <50 mg/dL (<2.8 mmol/L).

Hypoglycemic events should be appropriately recorded in the eCRF. The following hypoglycemia definition should be applied for reporting in the eCRF and evaluating hypoglycemic events;

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 PG to normal is considered sufficient evidence that the event was induced by a low PG (≤70 mg/dL [≤3.9 mmol/L]).

Documented symptomatic hypoglycemia: an event during which typical symptoms of hypoglycemia are accompanied by a measured PG concentration ≤70 mg/dL (≤3.9 mmol/L).

Asymptomatic hypoglycemia: an event not accompanied by typical symptoms of hypoglycemia but with a measured PG concentration ≤70 mg/dL (≤3.9 mmol/L).

Nocturnal hypoglycemia: is defined as any documented symptomatic hypoglycemic event that occurs between bedtime and waking.

Hypoglycemic events recorded by the euglycemic clamp device during the evaluation will not be documented. In the case of a hypoglycemic event (other than severe), the actual PG value, if measured, should be recorded in the eCRF alongside one of the above terms, together with any treatments administered and should not be recorded as an AE. Cases of hypoglycemia may be treated with foods or beverages rich in carbohydrate such as fruit, juice, skimmed milk, or energy bars.

All episodes of hypoglycemia that are determined by the investigator to constitute severe hypoglycemia according to the definition provided should be reported as SAEs.

10.4.5. Other Safety Measures

10.4.5.1. Physical Examination

Physical examinations and routine medical assessments will be conducted as specified in the Study Schedule and as clinically indicated (Attachment 1).

10.4.5.2. Vital Signs

Blood pressure and pulse rate will be measured as specified in the Study Schedule and as clinically indicated (Attachment 1).

Blood pressure and pulse rate should be measured after the subject has been supine for at least 5 minutes.

If orthostatic measurements are required, subjects should be supine for at least 5 minutes and stand for at least 3 minutes.

If the subject feels unable to stand, supine vital signs only will be recorded.

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 and agreed upon between the sponsor and investigator.

10.4.5.3. Body Weight

Body weight will be recorded as specified in the Study Schedule and as clinically indicated (Attachment 1).

Subjects should be weighed twice. The scale should be zeroed before each time the subject is weighed. Subjects should remove their shoes and any additional layers of clothing (such as sweaters or coats) and empty their pockets before being weighed. If possible, subjects should also be given the opportunity to empty bladder/bowel before being weighed. Both weight values should be recorded in the source documentation and the eCRF.

10.4.5.4. Electrocardiograms

For each subject, a single 12-lead digital ECG will be collected according to the Study Schedule (Attachment 1). Electrocardiograms must be recorded before collecting any blood for safety or PK tests. 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. Collection of additional ECGs at a particular time point is allowed to ensure high quality records. All ECGs recorded should be stored at the investigator site.

Electrocardiograms will be interpreted by a qualified physician (the investigator or qualified designee) at the site 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/QTc 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.

10.4.6. Safety Monitoring

The Lilly clinical pharmacologist 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 clinical pharmacologist or CRP will consult with the functionally independent Global Patient Safety

therapeutic area physician or clinical research scientist when appropriate, and periodically review:

- trends in safety data
- laboratory analytes
- AEs

10.4.7. Complaint Handling

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

Complaints related to unblinded comparator drugs or concomitant drugs/drug delivery systems (with the exception of the U-500 OmniPod) are reported directly to the manufacturers of those drugs/devices in accordance with the package insert.

The investigator or his/her designee is responsible for handling the following aspects of the product complaint process in accordance with the instructions provided for this study:

- recording a complete description of the product complaint reported and any associated AEs using the study-specific complaint forms provided for this purpose
- faxing the completed product complaint form within 24 hours to Lilly or its designee

If the investigator is asked to return the product for investigation, he/she will return a copy of the product complaint form with the product.

10.5. Appropriateness and Consistency of Measurements

All assessments made in this study are standard, widely used, and generally recognized as reliable, accurate, and relevant.

10.6. Compliance

Every attempt will be made to select subjects who have the ability to understand and comply with instructions. Noncompliant subjects may be discontinued from the study. The time and day of drug administration will be recorded. Drug accountability records will be maintained by the study site.

The specifications in this protocol for the timings of safety, PK, and PD sampling are given as targets, to be achieved within reasonable limits. Modifications may be made to the time points based upon the safety and PK information obtained. The scheduled time points may be subject to minor alterations; however, the actual time must be correctly recorded in the eCRF.

Any major modifications that might affect the conduct of the study, subject safety, and/or data integrity will be detailed in a protocol amendment.

11. Sample Size and Data Analyses

11.1. Determination of Sample Size

The primary objective of Part A of the study is to compare the t_{max} of U-500R administered by single SC injection or CSII. Up to 32 subjects may be enrolled to ensure that approximately 24 subjects complete the study.

The sample size is based on a calculation of precision of the t_{max}. Intra-subject variability for t_{max} has not been assessed previously. Based on an assumption of an intra-subject variability estimate of 20% for t_{max}, a sample size of 24 completed subjects in Part A will provide approximately 90% coverage probability that the half-width of the 90% confidence interval (CI) for the ratio of geometric means for t_{max} will be within 0.116 in the log scale, which corresponds to approximately 12% of the geometric mean ratio estimate in the natural scale.

If any subjects discontinue the study prior to completion of the 3 periods, the replacement subjects who are enrolled will assume the discontinued subjects' randomization schedule and will complete all 3 periods.

11.2. Data Analysis Plans

11.2.1. General Considerations

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

Pharmacokinetic and PD analyses will be conducted on the full analysis set. This set includes all data from all randomized subjects receiving at least 1 dose of the investigational product and having evaluable PK/PD data according to the treatment the subjects actually received. Safety analyses will be conducted for all enrolled subjects, regardless of whether they completed all protocol requirements.

Additional exploratory analyses of the data will be conducted as deemed appropriate. Analyses will be fully detailed in the statistical analysis plan. 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.

11.2.2. Study Participant Disposition

All subjects who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their discontinuation will be given.

11.2.3. Study Participant Characteristics

The subject's age, sex, weight, BMI, height, race/sub-race, smoking habits, and/or other demographic characteristics will be recorded.

11.2.4. Pharmacokinetic Analyses

11.2.4.1. Pharmacokinetic Parameter Estimation

Pharmacokinetic parameter estimates will be calculated by standard noncompartmental methods of analysis. C-peptide may be used to correct serum IRI concentrations for endogenous insulin.

The primary parameter for analysis in Part A will be t_{max}. Other noncompartmental parameters such as C_{max}, AUC from time zero to infinity (AUC[0-∞]), AUC(0-t_{last}), $t_{1/2}$, CL/F, and V_z/F may be reported, as appropriate.

The primary PK parameters for Part B will be AUC(0-24) and C_{max}; other PK parameters such as t_{max} and AUC of U-500R over each dosing interval and over each meal at steady state may also be reported, as appropriate.

Parameters will be individually calculated for each subject based on actual collection times.

11.2.4.2. Pharmacokinetic Statistical Inference

For Part A, the primary parameter for statistical analysis will be t_{max}. The values for t_{max} will be log-transformed and evaluated in a linear mixed-effects model with fixed effects for administration method, period and sequence (sequences 1 and 3, versus 2 and 4), and a random effect for subject. The difference in least squares (LS) means along with the 90% CI will be back-transformed to present the ratios of geometric means and the CI for comparison of the administration methods (single SC injection and CSII). This same analysis will be repeated for AUC(0-t_{last}), AUC(0-∞), and C_{max}.

The values of t_{max} will also be analyzed non-parametrically using a Wilcoxon signed-rank test. Median differences and approximate 90% CIs for the difference will be calculated for the comparisons of the administration methods (single SC injection and CSII).

No formal statistical analysis will be conducted for Part B; PK parameters will be listed and summarized.

Exploratory analyses may be performed for other PK parameters as deemed appropriate.

11.2.5. Pharmacodynamic Analyses

11.2.5.1. Pharmacodynamic Parameter Estimation

Glucose infusion rate and PG values will be recorded. Glucose infusion rates will be used to calculate PD parameters, including G_{tot}, R_{max}, tR_{max}, and tR_{last}. Other parameters, such as tR_{onset}, early tR_{max50}, and late tR_{max50}, may be reported. Parameters will be individually calculated for each subject and presented by summary statistics.

11.2.5.2. Pharmacodynamic Statistical Inference

For Part A, the values of tR_{max} will be analyzed non-parametrically using a Wilcoxon signed-rank test. Median differences and approximate 90% CIs for the difference will be calculated for the comparisons of the administration methods (single SC injection and CSII).

The PD parameters (R_{max} and G_{tot}) will be log-transformed prior to analysis and a linear mixed-effects model fitted to the data, with administration method, period, and sequence (sequences 1 and 3, versus 2 and 4) as fixed effects and subject as a random effect. For each parameter, the difference in LS means along with the 90% CI will be back-transformed to present the ratios of geometric means and the CI for comparison of the administration methods.

Other time parameters will be analyzed non-parametrically.

No formal statistical analysis will be conducted for Part B; PD parameters will be listed and summarized.

Exploratory analyses may be performed for other PD parameters as deemed appropriate.

11.2.6. Safety Analyses

11.2.6.1. Clinical Evaluation of Safety

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.

The incidence of symptoms for each treatment will be presented by severity and by association with investigational product, study procedure, or drug delivery device, as perceived by the investigator. Symptoms reported to occur prior to the first dose 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 regulatory dictionary.

The number of investigational product-related SAEs will be reported.

11.2.6.2. Statistical Evaluation of Safety

Safety parameters that will be assessed include safety laboratory parameters, vital signs, and ECG parameters. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

11.3. Interim Analyses

An interim analysis will be conducted after approximately 12 subjects have completed both periods of Part A of the study. An interim analysis of safety, PK and PD will be carried out and will be used to inform ongoing analysis of a Phase 3b trial, as appropriate.

12. Data Management Methods

12.1. 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.
- sponsor start-up training to instruct the investigators and study coordinators. This training will give instruction on the protocol, the completion of the eCRFs, 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 eCRF 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 study site. 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.

12.2. Data Capture Systems

12.2.1. Source Data and Case Report Form

A source document is the first record of data. These can be paper (for example, ECG tracing or subject diary), a paper CRF on which the data is initially recorded, or data captured directly on an investigator site electronic system (for example, Holter Monitor record data files or electronic health record). The site must retain all source records and must maintain a record of any data where source data are directly entered into the paper CRF.

Data may then be entered into either an electronic or paper CRF and the process will be documented and communicated by the sponsor to the investigator site before first subject visit.

Some investigator site data may be collected directly in the paper CRF whereas other data that are collected by the site on paper or electronic records may be transferred to the paper CRF.

Lilly does not allow direct source data entry into Lilly computer systems, with the exception of the investigator site systems at the Lilly CRU.

For data handled by a data management third party organization (TPO), CRF data and some or all data that are related will be managed and stored electronically in the TPO system. Subsequent to the final database lock, validated data will be transferred to the sponsor.

For data handled internally, CRF data and some or all data that are related will be managed by the sponsor and stored electronically in the sponsor's system.

12.2.2. Ancillary Data

Data managed by a central vendor will be stored electronically in the central laboratory's database system. Data will subsequently be transferred from the central vendor to the Lilly generic labs system / TPO's system.

Bioanalytical data will be stored electronically in the bioanalytical laboratory's database. Data will subsequently be transferred from the bioanalytical laboratory to the Lilly generic labs system / TPO's system.

Data from complaint forms submitted to Lilly will be encoded and stored in the global product complaint management system.

13. Informed Consent, Ethical Review, and Regulatory Considerations

13.1. Informed Consent

The investigator is responsible for ensuring that the subject understands the potential risks and benefits of participating in the study, including 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 trial.

The ICF will be used to explain the potential risks and benefits of study participation to the subject in simple terms before the subject is entered into the study, and to document that the subject is satisfied with his or her understanding of the potential risks and benefits of participating in the study and desires to participate in the study.

The investigator is ultimately responsible for ensuring that informed consent is given by each subject before the study is started. 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 the investigational product.

As used in this protocol, the term "informed consent" includes all consent and assent given by subjects or their legal representatives.

13.2. Ethical Review

Lilly or its representatives must approve all ICFs before they are used at investigative sites(s). All ICFs must be compliant with the International Conference on Harmonization (ICH) guideline on GCP.

The investigator must give assurance that the ERB was properly constituted and convened as required by 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 investigative site(s). The ERB(s) will review the protocol as required.

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

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

13.3. Regulatory Considerations

This study will be conducted in accordance with:

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

2) ICH GCP Guideline [E6]

3) applicable laws and regulations

The investigator or designee will promptly submit the protocol to applicable ERB(s).

Some of the obligations of the sponsor will be assigned to a TPO.

An identification code assigned by the investigator to each subject will be used in lieu of the subject's name to protect the subject's identity when reporting AEs and/or other trial-related data.

13.3.1. Investigator Information

Site-specific contact information may be provided in a separate document.

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

13.3.3. 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.

14. References

[ADA] American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2012;35(Suppl 1): S11-S63.

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Attachment 1. Protocol IBHG Study Schedule

Study Schedule Protocol B5K-EW-IBHG

		Periods 1 and 2[a]			Period 3					
	Screening	Days			Days				Follow-up	Comments
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
Informed consent	X									
Subject admission to CRU		X				X				
U-100, U-200, and/or U-300 insulin dosing	X	X		X				X		U-100, U-200, and/or U-300 dosing on Day -1 of Period 1 and 2 to end ≥15 h prior to U-500R bolus dosing. Transition to U-100, U-200, and/or U-300 insulin after Period 3 is per investigator judgment.
Subject discharge from CRU				X				X		Subjects will remain at the CRU until BG is stable according to investigator judgment.
Recording of U-100, U-200, and/or U-300 insulin TDD	X	X		X				X		Subjects will record their U-100, U-200, and/or U-300 insulin TDD throughout the study apart from during dosing with U-500R.
Type and dose of insulin use recorded									X	

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
Telephone visit for U-500R dose titration					X					Telephone visit for review of SMPG 2 to 3 days after end of Period 2. Additional visits may be conducted at the investigator's discretion.
Recording of U-500R doses					X					
Start of 4.25-U/hour U-500R infusion		X								Infusion to start 12 hours prior to bolus administration by CSII and continue throughout the clamp procedures.
Bolus U-500R administration			X							
TID or BID administration of U-500R					X					Dosing will begin following Period 2 and will stop at the end of the Period 3 clamp procedure.
Height	X									
Weight	X	X				X			X	Body weight should be recorded on Day -1 of all study periods.

		Periods 1 and 2[a]			Period 3					
	Screening	Days			Days				Follow-up	Comments
Procedure	Up to 28 days prior to Period 1	-1	1	2	-10[b] to -2	-1	1	2	7 to 28 days after discharge or early discontinuation	
Vital signs (supine) (hours relative to bolus/morning dose)	X	-12 h (predose)[d]	Predose, 1, 2, 4, 8, 12, 16 h	24 h[c]			Predose, 1, 2, 4, 8, 12, 16 h	24 h	X	Supine blood pressure and/or pulse rate may be measured as clinically indicated. Additional vital signs may be measured during each study period, if warranted and agreed upon between Lilly and the investigator.
Clinical laboratory tests	X	X (Period 1 only)							X	See Attachment 2, Clinical Laboratory Tests, for details.
Pregnancy test	X	X				X			X	Serum pregnancy test will be performed at screening. Urine pregnancy test will be performed at every admission period and at poststudy, if applicable.
Physical exam	X									After screening, medical assessment only performed to include medical review and targeted examination, as appropriate.
12-lead ECG	X		Predose	24 h[c]			Predose	24 h	X	

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
SMPG	X									4-point SMPG to be conducted daily throughout the study, except whilst resident in the CRU. Mean SMPG for the 5 days prior to Period 3 will be collected in the eCRF.
Overnight PG stabilization		X				X				PG stabilized using an insulin lispro and/or glucose drip.
Euglycemic clamp			X				X			
Pharmacogenetic sample		X (predose)								Single sample for pharmacogenetic analysis taken on Day -1 of Period 1.
PK and C-peptide samples		-12, -8, -4 h	-0.5, 0 (predose), 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16 h	24 h			-0.5, 0 (predose), 0.5, 1, 2, 3, 4, 5 (predose for TID), 6, 7, 9, 11 (predose), 12, 13, 15, 18 h	24 h,		Sampling times are relative to the time of the start of study treatment morning bolus administration (0 h). Sampling times for Period 3 are relative to the time of breakfast (0 hr), lunch (5 hr) and dinner (11 hr).

Abbreviations: BG = blood glucose; BID = twice daily; CRU = clinical research unit; CSII = continuous subcutaneous insulin infusion; ECG = electrocardiogram; PG = plasma glucose; PK = pharmacokinetics; SMPG = self-monitored plasma glucose; TDD = total daily dose; TID = thrice daily; U-500R = human regular U-500 insulin.

[a] There may be 7 to 21 days between bolus doses in Period 1 and Period 2.

[b] There may be 5 to 10 days between the end of Period 2 and clamp initiation in Period 3 (Day 1), that is BID or TID dosing may begin on Day -10 through Day -5.

[c] Clamp procedure in Periods 1 and 2 are of a maximum duration of 24 hours. Vital signs and ECGs will be conducted at the end of the clamp procedure.

[d] CSII period only, taken prior to initiation of 12-hour U-500R infusion.

Attachment 2. Protocol IBHG Clinical Laboratory Tests

Laboratory Tests

Hematology:[a]	Clinical Chemistry[a]
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count	Bicarbonate
Mean cell volume	Chloride
Mean cell hemoglobin	Calcium
Mean cell hemoglobin concentration	Phosphorus
Leukocytes	Glucose fasting
Cell Morphology	Blood urea nitrogen
Absolute counts of:	Uric acid
Neutrophils	Total cholesterol
Lymphocytes	Triglycerides
Monocytes	High-density lipoprotein cholesterol
Eosinophils	Total protein
Basophils	Albumin
Platelets	Total bilirubin
Urinalysis[a]	Alkaline phosphatase
Specific gravity	Aspartate aminotransferase (AST)
pH	Alanine aminotransferase (ALT)
Protein	Creatinine
Glucose	Gamma-glutamyl transferase (GGT)
Ketones	PT/INR[b]
Bilirubin	
Urobilinogen	Ethanol testing[b,c]
Blood	Urine drug screen[b,c]
Nitrite	Hepatitis B surface antigen[b]
Microscopic examination of sediment[d]	Hepatitis C antibody[b]
	HIV[b]
	Pregnancy test[c]
	FSH[b]
	HbA1c

Abbreviations: CRU = clinical research unit; FSH = follicle-stimulating hormone; HbA1c = hemoglobin A1c; HIV = human immunodeficiency virus; INR = international normalized ratio; PT = prothrombin time.

a Laboratory tests will be performed at a local laboratory and results will be validated at the time of initial testing.

b Performed at screening only.

c A serum pregnancy test will be performed at screening and urine pregnancy tests will be conducted at each CRU admission for women of child-bearing potential. Urine drug screen and ethanol level may be repeated prior to admission to the CRU. These tests will not be required at the follow-up visit.

d Test only if dipstick result is abnormal.

Attachment 3. Protocol IBHG 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. Fewer venipunctures and blood draws may actually occur, but this will not require a protocol amendment.

Protocol B5K-EW-IBHG Sampling Summary

Purpose	Maximum Blood Volume per Sample (mL)	Maximum Number of Blood Samples	Maximum Total Volume (mL)
Screening tests[a]	22.2	1	22.2
Clinical laboratory tests[a]	12.5	2	25
Self-monitored plasma glucose	0.05	372[b]	18.6
Pharmacokinetics	3.5	51	178.5
C-peptide	2.5	51	127.5
Euglycemic clamp	3 mL per hour	24 hours x 3 periods	216
Pharmacogenetics	10	1	10
Total			597.8
Total for clinical purposes [rounded up to nearest 10 mL]			600

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

[b] Includes 4 point self-monitored plasma glucose throughout study and 8 possible 03:00 hours measurements during titration.

Attachment 4. World Health Organization (WHO) Classification Of Diabetes

Type 1 Diabetes Mellitus: Type 1 diabetes mellitus is judged to be present when the classical symptoms of diabetes (thirst, polyuria, wasting and stupor, or coma) are associated with readily detectable concentrations of glucose and ketone bodies in the blood and urine. Insulin treatment is necessary not only to control hyperglycemia, but also to prevent spontaneous ketosis and death.

Type 2 Diabetes Mellitus: Type 2 diabetes mellitus, although often asymptomatic, may also present with classical hyperglycemic symptoms (thirst, polyuria, weight loss), but despite hyperglycemia, ketone bodies are present in only low concentrations in the blood and urine. Coma is rare in type 2 diabetes, but may result from extreme hyperglycemia and hyperosmolarity; lactic acidosis or ketoacidosis can also occur in fulminating illness (for example, severe infection or mesenteric artery thrombosis) due to an acute increase in insulin requirements, but spontaneous ketosis does not occur. Some patients with type 2 diabetes later progress to a state of absolute insulin deficiency.

References:

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Attachment 5. Protocol Amendment B5K-EW-IBHG(b) Summary Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus

Overview

Protocol B5K-EW-IBHG Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated Subjects with Type 2 Diabetes Mellitus has been amended. The new protocol is indicated by Amendment (b) 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 inclusion criteria for BMI has been changed to ≥27.0 as the majority of subjects with T2DM are overweight or obese and this will aid recruitment whilst retaining a clinically relevant study population.
- The inclusion criteria for prestudy insulin has been amended to include U-200 and/or U-300 insulins (as these formulations are used by insulin resistant subjects with T2DM and formulations of different strength may be used in basal/bolus combinations) and acceptable prestudy insulin use has been amended to TDD ≥150 U OR single doses >100 U as part of an MDI regimen AND TDD ≤3.0 U/kg in order to aid recruitment, whilst still including subjects who are considered insulin resistant.
- Acceptable concomitant medication has been amended to include SGLT2 inhibitors in order to aid recruitment as these agents are used by insulin resistant patients with T2DM.
- The Abbreviations and Definitions section has been amended to include a definition for SGLT2.
- Several editorial updates have been made to take into account the allowance of U-200 and/or U-300 prestudy insulins and the removal of the requirements for subjects to be obese and for insulin resistance to be severe.

Revised Protocol Sections

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

1. Protocol B5K-EW-IBHG(b)

Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated~~, Obese~~ Subjects with Type 2 Diabetes Mellitus

2. Synopsis

Title of study: Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated~~, Obese~~ Subjects with Type 2 Diabetes Mellitus

Objectives: The primary objective of Part A is to compare the time of maximum drug concentration (t_{max}) of a 100-U bolus of human regular U-500 insulin (U-500R) (500 U/mL) administered via single subcutaneous (SC) injection versus a 100-U bolus of U-500R administered via continuous SC insulin infusion (CSII) in high-dose insulin-treated~~, obese~~ subjects with type 2 diabetes mellitus (T2DM).

The primary objective of Part B is to evaluate the area under the concentration versus time curve (AUC) from time zero to 24 hours postdose (AUC[0-24 hr]), maximum drug concentration (C_{max}), and maximum glucose infusion rate (GIR) (R_{max}) of U-500R at steady state following thrice- (TID) or twice- (BID) daily SC dosing in the study population.

Diagnosis and Main Criteria for Inclusion and Exclusion: High-dose insulin-treated (U-100, U-200, and/or U-300 insulin total daily dose [TDD] ≥~~>200~~150 U/day OR at least 1 dose >100 U as part of a multiple daily injection (MDI) regimen AND have TDD ≤3.0 U/Kg) males or females with T2DM, ≥18 to <75 years old, with a body mass index of ≥~~30~~27.0 kg/m^2, and HbA1c 7.5% to 11.5%, inclusive, at screening.

3. Table of Contents

Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated~~, Obese~~ Subjects with Type 2 Diabetes Mellitus

4. Abbreviations and Definitions

SGLT2 sodium-glucose co-transporter-2

Comparative Pharmacokinetics and Pharmacodynamics of Human Regular U-500 Insulin Administered Subcutaneously as a Bolus via Syringe versus Continuous Subcutaneous Insulin Infusion and Characterization of TID and BID Dosing at Steady State in High-Dose Insulin-Treated~~, Obese~~ Subjects with Type 2 Diabetes Mellitus

5. Introduction

5.2 Rationale and Justification for the Study

There have been no state-of-the-art euglycemic clamp PK/PD studies performed on U-500R in the target population of high-dose insulin-treated ~~obese~~ subjects with T2DM, in whom U-500R is most often used (Lane et al. 2009; Reutrakul et al. 2012; Jones and Idris 2013; Eby et al. 2013; Eby et al. 2014; Cochran et al. 2014). Likewise, there have been no PK/PD studies conducted on steady state time-concentration and time-action characteristics of U-500R on any population of subjects. Rigorous study in both of these settings will address major gaps in scientific knowledge and will further inform clinicians regarding appropriate use of U-500R in ~~severely~~ insulin-resistant patients with diabetes requiring high doses of insulin.

6.1 Primary Objective

Part A: to compare the t_{max} of a 100-U bolus of U-500R (500 U/mL) administered via single SC injection versus a 100-U bolus of U-500R administered via CSII in high-dose insulin-treated~~, obese~~ subjects with T2DM.

7.1 Summary of Study Design

This is a 2-part, Phase 1 (postmarketing), single-center, 3-period, 4-sequence, randomized, open-label, crossover euglycemic clamp study in high-dose insulin-treated~~, obese~~ subjects with T2DM. Part A (Periods 1 and 2) will compare the PK and PD of a 100-U bolus of U-500R administered via single SC injection versus a 100-U bolus of U-500R administered via CSII.

Subjects completing Part A will transition to Part B (Period 3), which will evaluate the PK and PD characteristics of U-500R administered by TID and BID MDIs under steady-state conditions.

7.1.2 Part A (Study Periods 1 and 2)

Subjects will be admitted to the clinical research unit (CRU) on Day -1 of each period and be fasted from 12 hours prior to clamp initiation until completion of the clamp procedure.

All subjects will stop their prestudy U-100, U-200, and/or U-300 insulin dosing at least 15 hours prior to U-500R bolus dosing. Subjects randomized to receive U-500R by SC injection will receive a 100-U U-500R bolus administration by SC injection prior to initiation of the clamp. Subjects randomized to receive U-500R by CSII will have a U-500 OmniPod Insulin Management System (U-500 OmniPod) fitted by a qualified member of CRU staff, which will administer a 12-hour infusion of 4.25 U/h of U-500R, prior to bolus administration of 100 U of U-500R (via the same pump) and initiation of the clamp. The 4.25-U/h U-500R infusion will continue throughout the duration of the clamp following bolus administration by the U-500 OmniPod.

Overnight, between Day -1 and Day 1, all subjects will have their PG stabilized, using an insulin lispro and/or glucose drip, to a target fasting PG concentration of 120 mg/dL (6.7 mmol/L) (with an acceptable range of 100 to 130 mg/dL [5.6 to 7.2 mmol/L]).

Following bolus dose administration (or initiation of bolus dose administration for CSII), subjects will undergo a euglycemic clamp with a maximum duration of 24 hours. Following completion of the clamp procedures, subjects will be provided a meal and will be medically assessed and discharged from the CRU, unless the investigator has any safety concerns. If glucose is still being infused at the time of clamp discontinuation, the subject will be monitored for safety and will remain in the CRU until stable according to investigator judgment (see Section 10.4.1).

Blood samples will be collected prior to and during the clamp procedure (see Attachment 1) to determine serum immunoreactive insulin (IRI) and C-peptide concentrations for PK and PD analysis.

Following termination of the clamp procedure in Period 1, subjects will resume their prestudy treatment regimen (which may include basal; premixed; and/or basal/bolus U-100, U-200, and/or U-300 insulin regimens with or without antihyperglycemic agents [AHAs] as listed in Inclusion Criterion [5], Section 8.1.1) until their admission to the CRU on Day -1 of Period 2. There will be a washout period of 7 to 21 days between U-500R bolus doses in Periods 1 and 2.

Following termination of the clamp procedure in Period 2, subjects will be instructed on administration of U-500R either by TID or BID MDIs as described in Section 7.1.3 and Section 9.4. There will be a period of 5 to 10 days between the end of the clamp in Period 2 and the clamp procedure in Period 3 to allow U-500R concentrations to reach steady state.

7.1.3 Part B (Study Period 3)

Following termination of the clamp procedure in Period 2, subjects will transition, as randomized, to TID or BID MDIs of U-500R. Human regular U-500 insulin will be self-administered by the subjects at home under telephone guidance from CRU staff during a telephone visit 2 to 3 days after the end of Period 2 (additional telephone or office visits may be added at the investigator's discretion). Initial U-500R doses will be based on each subject's ~~U-100~~ insulin TDD prior to admission for Period 2, with titration for safety, if necessary, using the formulas given in Section 9.4. There will be 5 to 10 days between the end of Period 2 and the clamp in Period 3 (that is, dosing will begin on up to Day -10 and no later than Day -5 of Period 3) to allow steady state of the U-500R dosing regimen to be achieved. Mean self-monitored plasma glucose (SMPG) will be recorded from Day -5 until admission to the CRU.

Subjects will be admitted to the CRU on Day -1 of Period 3, prior to their pre-evening meal dose, which will be administered by CRU staff, and undergo fasting, PG stabilization, and a 24-hour euglycemic clamp as detailed in Section 7.1.2, with PK sampling as detailed in the Study Schedule (Attachment 1).

Following termination of the clamp procedure in Period 3 (Day 2), subjects will resume their prestudy ~~U-100~~ insulin treatment regime. Discharge from the CRU will be subject to the same postclamp safety assessments as detailed in Section 7.1.2 and Section 10.4.1. Follow-up will occur 7 to 28 days after the end of Period 3 when safety procedures will be conducted and insulin use and dose will be recorded as detailed in the Study Schedule (Attachment 1).

7.2 Discussion of Design and Control

In Part A of this study, a 2-period, randomized, crossover design has been chosen to allow each subject to act as his/her own control. Prestudy U-100, U-200, and/or U-300 insulin will be taken at the same dose and at approximately the same time each day prior to Period 1 and between Periods 1 and 2, and stopped at least 15 hours prior to U-500R bolus administration in Part A in order to reduce variation in insulin carryover (as the bioanalytical methodology is not specific to U-500R).

The use of a euglycemic clamp technique will provide data on the glucodynamic activity of U-500R. This is standard methodology routinely used to safely measure insulin action in clinical studies.

High-dose insulin-treated~~, obese~~ subjects with T2DM are representative of the population that is prescribed the U-500R formulation. An open-label design is considered appropriate as the PK/PD parameters being investigated in this study are objective endpoints.

Fasting and pre-meal plasma-equivalent glucose target ranges in Period 3 are 71 to 130 mg/dL (3.9 to 7.2 mmol/L), consistent with American Diabetes Association (ADA) standards of care (ADA 2012).

8.1.1 Inclusion Criteria

[3] have a BMI of ≥~~30~~27.0 kg/m^2 at screening

[4] are current users of U-100, U-200, and/or U-300 insulin/analog as basal, premixed, and/or basal/bolus delivered with any injection device (pens and/or syringe/vial but excluding CSII/insulin pump use in the preceding 3 months), taking:

~~with~~ a TDD of ≥~~>200~~ 150 U ~~AND~~

OR ~~have TDD ≤3.0 U/Kg~~

at least 1 dose >100 U as part of an MDI regimen~~, basal, premixed, or basal/bolus by any injection device (pens and/or syringe/vial but excluding CSII/insulin pump use in the preceding 3 months)~~

AND

TDD ≤3.0 U/kg

[5] Concomitant AHA therapy may include:

- metformin (MET)~~,~~
- dipeptidyl peptidase-4 inhibitors~~,~~
- pioglitazone (doses ≤30 mg/day)~~, and/or~~
- GLP-1 receptor agonists
- sodium-glucose co-transporter-2 (SGLT2) inhibitors, except in combination with GLP-1 receptor agonists (see Exclusion Criterion [31] for excluded AHA therapy).
  Subjects' AHA therapy must have been stable for ≥3 months (except for weekly GLP-1 receptor agonists which must have been stable for ≥4 months).

[9] must be able to safely withhold administration of U-100, U-200, and/or U-300 insulins from ≥15 hours prior to clamp procedures in Periods 1 and 2 as applicable, in the judgment of the investigator

8.1.2. Exclusion Criteria

[31] have used ~~sodium-glucose co-transporters inhibitors,~~ rosiglitazone, pramlintide, once-weekly or BID exenatide, or other injectable or oral AHA not listed in Inclusion Criterion [5] in the 3 months prior to screening; or are taking oral antidiabetic medications at doses exceeding the respective product labels; or have a contraindication to current oral antidiabetic medication usage per the respective product labels (for example, MET: serum creatinine ≥1.4 mg/dL in women or ≥1.5 mg/dL in men).

9.2. Treatment Administration

The investigator or designee is responsible for:

- explaining the correct use of the investigational product to the subjects and site personnel,

- verifying that instructions are followed properly,
- maintaining accurate records of investigational product dispensing and collection,
- and returning all unused medication to Lilly or its designee at the end of the study.

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

Subjects will be instructed to contact the investigator as soon as possible if he or she has a complaint or problem with the investigational product or drug delivery system so that the situation can be assessed.

All clinical trial material provided to the investigator will be stored in a secure place and allocated and dispensed by appropriately trained persons. The allocation and dispensing of the investigational products will be fully documented and verified by a second person. Detailed records of the amounts of the investigational product received, dispensed, and remaining at the end of the study will be maintained. Not in-use or unopened Humulin R U-500 vials should be stored in a refrigerator, (2°C to 8°C [36° F to 46°F]), but not in the freezer. Once opened, the U-500R vial can be kept unrefrigerated as long as it is kept as cool as possible (below 30°C [86°F]) and away from heat and light. In-use vials must be used within 40 days, even if they still contain U-500R (Humulin R U-500 Package Insert, 2014). Clinical trial materials will be labeled according to the respective country's regulatory requirements.

During periods of prestudy U-100, U-200, and/or U-300 insulin treatment, subjects will self-administer their ~~U-100~~ insulin at home, at approximately the same time each day. The last dose prior to assessment should be taken at approximately the same time in each period and will be at least 15 hours before the U-500R bolus dose and clamp procedure in Part A. The ~~U-100~~ insulin TDD should remain approximately the same throughout Part A of the study (except while subjects are resident in the CRU when it will not be taken).

9.3 Rationale for Selection of Dose

A dose of 100 U of U-500R was chosen for Part A of this study as this is within the range commonly encountered in high-dose insulin-treated patients whose T2DM is managed with U-500R either by MDI or off-label via CSII (Lane et al. 2009; de la Peña et al. 2011; Reutrakul et al. 2012; Cochran et al. 2014; and Lane et al. 2013).

Study dosing and conversion and titration regimens for the TID and BID treatment groups in Part B, both guided by pre-Period 2 U-100, U-200, and/or U-300 insulin TDD (based on the highest TDD recorded in the 3 days prior to Period 2 which should be approximately unchanged from pre-Period 1) and baseline HbA1c (screening), and subsequent glucose monitoring, respectively, are based on literature expert reviews (Lane et al. 2009; Segal et al. 2010; Reutrakul, et al. 2012) and on the results from simulations performed using a systems physiology model of diabetes (in Physiolab®; de la Peña et al. 2014) based on data from previous PK/PD studies of

U-500R and U-100 human regular insulin (de la Peña et al. 2011; Studies F3Z-LC-IMAC and H7U-MC-IDBM).

9.4 Dose Conversion from Prestudy Insulin in Part B

Subjects will record their ~~U-100~~ insulin doses throughout the study. Baseline U-100, U-200, and/or U-300 insulin TDD will be defined as the highest TDD recorded in the 3 days prior to Period 2. All U-100, U-200, and/or U-300 insulin therapy will be discontinued upon initiation of study drug, but permitted prestudy AHA therapy should continue to be taken at prestudy doses, except on clamp assessment days. For all subjects with HbA1c >8.0% at screening, initial U-500R TDD will be the same (in units) as U-100, U-200, and/or U-300 insulin TDD at screening.

For all subjects with baseline HbA1c ≤8.0% at screening, initial U-500R TDD dose will be reduced by 20% from the U-100, U-200, and/or U-300 insulin TDD at screening.

9.7 Concomitant Therapy

Subjects on stable concomitant medication at the time of study entry should continue their regular, unchanged dose throughout the study; for example, hormonal contraceptives, thyroid replacement therapy, anti-hypertensives, low-dose aspirin, and cholesterol lowering agents. Prescription medications (other than those allowed in Inclusion Criteria [4] and [5]), herbal medications, or nutritional supplements that affect PG or insulin sensitivity, or promote weight loss will not be allowed within 4 weeks prior to dosing. Initiation of new over-the-counter medication within 7 days prior to dosing, or prescription medication or herbal medication within 14 days prior to dosing may be permitted following discussion with the Lilly clinical pharmacologist. Vitamin/mineral supplements and/or occasional use of acetaminophen will be allowed.

Subjects may continue to take their stable prestudy AHA dose (MET, dipeptidyl peptidase-4 inhibitors, pioglitazone [doses ≤30 mg/day], ~~and/or~~ GLP-1 receptor agonists, and/or SGLT2 inhibitors [not in combination with GLP-1 receptor agonists]), unchanged, throughout the study, except on clamp assessment days.

Rosiglitazone, pramlintide, once weekly or BID exenatide~~, sodium-glucose co-transport inhibitors~~, other injectable or oral AHAs not listed in Inclusion Criterion [5], or permitted AHAs taken at doses other than that prescribed by the product label will not be allowed.

Attachment 1. Protocol IBHG Study Schedule

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
Informed consent	X									
Subject admission to CRU		X				X				
U-100, U-200, and/or U-300 insulin dosing	X	X		X				X		U-100, U-200, and/or U-300 dosing on Day -1 of Period 1 and 2 to end ≥15 h prior to U-500R bolus dosing. Transition to U-100, U-200, and/or U-300 insulin after Period 3 is per investigator judgment.
Subject discharge from CRU				X				X		Subjects will remain at the CRU until BG is stable according to investigator judgment.
Recording of U-100, U-200, and/or U-300 insulin TDD	X	X		X				X		Subjects will record their U-100, U-200, and/or U-300 insulin TDD throughout the study apart from during dosing with U-500R.
Type and dose of insulin use recorded									X	

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
Telephone visit for U-500R dose titration					X					Telephone visit for review of SMPG 2 to 3 days after end of Period 2. Additional visits may be conducted at the investigator's discretion.
Recording of U-500R doses					X					
Start of 4.25-U/hour U-500R infusion		X								Infusion to start 12 hours prior to bolus administration by CSII and continue throughout the clamp procedures.
Bolus U-500R administration			X							
TID or BID administration of U-500R					X					Dosing will begin following Period 2 and will stop at the end of the Period 3 clamp procedure.
Height	X									
Weight	X	X				X			X	Body weight should be recorded on Day -1 of all study periods.

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
Vital signs (supine) (hours relative to bolus/morning dose)	X	-12 h (predose)[d]	Predose, 1, 2, 4, 8, 12, 16 h	24 h[c]			Predose, 1, 2, 4, 8, 12, 16 h	24 h	X	Supine blood pressure and/or pulse rate may be measured as clinically indicated. Additional vital signs may be measured during each study period, if warranted and agreed upon between Lilly and the investigator.
Clinical laboratory tests	X	X (Period 1 only)							X	See Attachment 2, Clinical Laboratory Tests, for details.
Pregnancy test	X	X				X			X	Serum pregnancy test will be performed at screening. Urine pregnancy test will be performed at every admission period and at poststudy, if applicable.
Physical exam	X									After screening, medical assessment only performed to include medical review and targeted examination, as appropriate.
12-lead ECG	X		Predose	24 h[c]			Predose	24 h	X	

		Periods 1 and 2[a]			Period 3					
	Screening	**Days**			**Days**				**Follow-up**	**Comments**
Procedure	**Up to 28 days prior to Period 1**	**-1**	**1**	**2**	**-10[b] to -2**	**-1**	**1**	**2**	**7 to 28 days after discharge or early discontinuation**	
SMPG	X									4-point SMPG to be conducted daily throughout the study, except whilst resident in the CRU. Mean SMPG for the 5 days prior to Period 3 will be collected in the eCRF.
Overnight PG stabilization		X				X				PG stabilized using an insulin lispro and/or glucose drip.
Euglycemic clamp			X				X			
Pharmacogenetic sample		X (predose)								Single sample for pharmacogenetic analysis taken on Day -1 of Period 1.
PK and C-peptide samples		-12, -8, -4 h	-0.5, 0 (predose), 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16 h	24 h			-0.5, 0 (predose), 0.5, 1, 2, 3, 4, 5 (predose for TID), 6, 7, 9, 11 (predose), 12, 13, 15, 18 h	24 h,		Sampling times are relative to the time of the start of study treatment morning bolus administration (0 h). Sampling times for Period 3 are relative to the time of breakfast (0 hr), lunch (5 hr) and dinner (11 hr).

Abbreviations: BG = blood glucose; BID = twice daily; CRU = clinical research unit; CSII = continuous subcutaneous insulin infusion; ECG = electrocardiogram; PG = plasma glucose; PK = pharmacokinetics; SMPG = self-monitored plasma glucose; TDD = total daily dose; TID = thrice daily; U-500R = human regular U-500 insulin.

[a] There may be 7 to 21 days between bolus doses in Period 1 and Period 2.

[b] There may be 5 to 10 days between the end of Period 2 and clamp initiation in Period 3 (Day 1), that is BID or TID dosing may begin on Day -10 through Day -5.

[c] Clamp procedure in Periods 1 and 2 are of a maximum duration of 24 hours. Vital signs and ECGs will be conducted at the end of the clamp procedure.

[d] CSII period only, taken prior to initiation of 12-hour U-500R infusion.