Statistical Analysis Plan

Protocol No.: API-I004-CL-B

Title: Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) Biosimilarity of Proposed Biosimilar Rapid-Acting Insulin Aspart (I004) and NovoLog® after Single-Dose Subcutaneous Administration to Healthy Volunteers

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STATISTICAL ANALYSIS PLAN

Protocol No. API-I004-CL-B

Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) Biosimilarity of Proposed Biosimilar and Interchangeable Rapid-Acting Insulin Aspart (I004) and Novolog® after Single-Dose Subcutaneous Administration to Healthy Volunteers

(A Single-Center, Randomized, Double-Blinded, Two-Treatment
Two-Period, Two-Sequence, Crossover, Hyperinsulinemia-Euglycemic Clamp Study)

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ATTACHMENTS Template of Statistical Analysis Output for Clinical Study Protocol No. API-I004-CL-B (Available Upon Request)

List of Abbreviations

A - K		L - Z	
AE	Adverse event	Lb	Pound
ADE	Adverse drug event	LC/MS/MS	Liquid Chromatography with tandem mass spectrometry
ANOVA	Analysis of variance	LOESS	Locally estimated scatterplot smoothing
AUC	Area under the concentration-time curve	MedDRA	Medical Dictionary for Regulatory Activities
AUC_{GIR}	Area under the glucose infusion rate-time curve	Mg	Milligram
BE	Bioequivalence	MRB	Management Review Board
BLA	Biologic License Application	MSE	Mean square error
BMI	Body mass index	PD	Pharmacodynamics
CI	Confidence interval	PK	Pharmacokinetics
CL/F	Apparent clearance	PT	Preferred Terminology
C_{max}	Maximum concentration	PPP	Per-Protocol Population
CRF	Case report form	QA	Quality Assurance
CV	Coefficient of variation	QL	Qualitative limit
DBP	Diastolic Blood Pressure	R&D	Research and Development
DFT	Deviation from the Target	rDNA	Recombinant deoxyribonucleic acid
ECG	Electrocardiogram	RP	Reference Product
eCRF	Electronic case report form	SAP	Statistical analysis plan
FDA	Food and Drug Administration	SAS/STAT®	State-of-the-art statistical analysis software
FRD	Final Reported Data	SBP	Systolic Blood Pressure
GIR	Glucose infusion rate	SC	Subcutaneous
HbA1C	Glycosylated hemoglobin	SOP's	Amphastar standard operating procedures
HI	Human Insulin	SPD	Sever Protocol Deviation
HIV	Human immunodeficiency virus	SSE	Sum of square error
HR	Heart Rate	t½	Terminal insulin half-life
IA	Insulin Aspart	$tGIR_{max}$	Time to maximum glucose infusion rate
ICH	International Conference on Harmonization	t_{max}	Time to maximum serum insulin concentration (in concentration time curve)
IP	Investigational Product	TP	Treated Population
ITD	Initially Tested Data	TSH	Thyroid-stimulating hormone
ITT	Intent to Treat	UA	Urinalysis
IV	Intravenous	Vz/F	Apparent volume of distribution at terminal phase
Kg	Kilogram	YSI	YSI 2300 STAT Glucose Analyzer

1. INTRODUCTION

1.1 BACKGROUND[1]

In 2015, diabetes affected 30.3 million Americans or 9.4% of the total US population, including 7.2 million people who were undiagnosed. Recently, there have been dramatic annually increases in the incidence of diabetes, and should this upward trend continue, the total prevalence of diagnosed and undiagnosed diabetes in the US adult population is projected to increase to as much as 33% by the year 2050.

The results of the Diabetes Control and Complication Trial (DCCT) demonstrated that tight glycemic control can prevent or reduce the microvascular complications of diabetes, and such as retinopathy, nephropathy, neuropathy as well as reduce cardiovascular–related mortality. To optimize blood glucose control in patients, intensive insulin regimens are used, although current replacement insulin formulations only approximate physiologic insulin secretion, resulting in high postprandial blood glucose peaks.

Another disadvantage of intensive insulin regimens is the risk of hypoglycemia. The development of rapid-acting insulins with accelerated absorption has improved the postprandial glucose excursions and allowed for more precise dosing.

The development of rapid-acting insulin analogs with fast absorption has reduced postprandial glucose excursions and has allowed greater flexibility in insulin therapy as well as a more physiological insulin replacement with more precise dosing regimens.

Insulin Aspart [rDNA origin] injection, a rapid-acting human insulin analog, is developed by Amphastar, which is denoted as I004. The reference product (RP) of I004 is NovoLog®.

I004 and NovoLog® are homologous with regular human insulin, with the exception of a single substitution of the amino acid proline by aspartic acid in position B28, and are produced by recombinant DNA technology. The insulin formulation of I004 is available in a concentration corresponding to 100 units/mL (U-100).

Please refer to the Investigator's Brochure /Package Insert for further information on NovoLog®.

1.2 RATIONALE FOR THE PROPOSED STUDY

This clinical study, API-I004-CL-B, is a single-center, randomized, double-blinded, two-treatment, two-period, two-sequence, crossover, hyperinsulinemia-euglycemic clamp study. This

study is designed to evaluate the PK and PD profile required to support the future Biologic License Application (BLA) for biosimilar interchangeable use of I004. Therefore, the comparison of rapid-acting insulin I004 and Novolog with regard to the AUC_{IA} (0-12h), area under the serum insulin profile for the drug, and AUC_{GIR} (0-12h), area under the glucose infusion rate as primary endpoints, will be performed.

Safety, cardiac safety (ECG), and tolerability after SC administration will also be determined.

1.3 SUMMARY OF PRE-CLINICAL /CLINICAL STUDIES

Information regarding characterization, structure and properties of Insulin aspart [rDNA origin] are provided in the Novolog® Investigator's Brochure (IB).

For data of in vitro and in vivo pharmacologic and toxicologic studies and clinical studies, reference is made to [redacted] *(Novolog®,* [redacted]*).* The sponsor refers to the information and FDA safety determinations in the approved product labeling for these products.

Novolog will be commercially procured from the US market and will be used as the investigational product and comparator in this study. It will be administered at the same dose and in the same fashion, consistent with the approved product information.

[redacted] This clinical study I004-CL-B (proposed n=60) is a pivotal PK/PD study.

1.4 RATIONALE FOR TREATMENT AND DOSE

The dose of 0.2 units/kg body weight has been chosen for this study in order to provide a robust dose-response relationship relevant for healthy subjects. In addition, the dose used is representative of doses in healthy subjects and is within the dose range used in other clinical glucose clamp studies conducted to date, where no safety concerns have been observed at this dose level.

2. OBJECTIVES

The goal of this pivotal study is to determine PK/PD biosimilarity between the I004 and NovoLog® through assessment of *in vivo* PK and PD in approximately 60 healthy adult volunteers.

2.1 PRIMARY OBJECTIVES

To assess and compare the PK profile of I004 and Novolog by:

(i) Maximum insulin concentration, C_{IAmax}
(ii) Area under the insulin concentration-time curve, $AUC_{IA\ (0\text{-}12h)}$

To assess and compare the PD profile of I004 and Novolog by:

(i) Maximum glucose infusion rate, G_{max}
(ii) Area under the curve for the glucose infusion rate (GIR)-time curve from administration to end of clamp time, $AUC_{G\ (0\text{-}12h)}$

2.2 SECONDARY OBJECTIVES

To assess and compare the PK profile of I004 and Novolog by:

(i) Partial AUC_{IA}, e.g., $AUC_{IA\ (0\text{-}2h)}$
(ii) Area under the insulin concentration curve extrapolated to infinite time, $AUC_{IA(0\text{-}\infty)}$
(iii) Time until C_{max} is reached, t_{IAmax}
(iv) Apparent clearance, CL/F
(v) Apparent volume of distribution, Vz/F
(vi) Apparent terminal half-life, $t_{1/2}$

To assess and compare the PD profile of I004 and Novolog by:

(i) Total and partial AUC of GIR, e.g., $AUC_{G(0\text{-}last)}$, $AUC_{GIR(0\text{-}2h)}$
(ii) Value of last measurable GIR, G_{last}
(iii) Time until maximum glucose infusion rate is reached, t_{Gmax}
(iv) Time of start of GIR post-dose, t_{Gonset}
(v) Time of last measurable GIR, t_{Glast}
(vi) $t\text{-}_{G50\%early}$, defined as the time to 50% maximal GIR before t_{Gmax}
(vii) $t\text{-}_{G50\%late}$, defined as the time to 50% maximal GIR after t_{Gmax}

To assess the confounding effect of endogenous human insulin:

(i) PK profile of endogenous human insulin

2.3 SAFETY OBJECTIVES

To assess safety and tolerability of I004 and Novolog by:

(i) Incidence of adverse events (including hypoglycemia)
(ii) Clinical findings on physical examination
(iii) Clinical laboratory parameters (hematology, serum chemistry and urinalysis)
(iv) Vital signs (blood pressure, temperature, respiratory rate, and heart rate) measurements
(v) 12-lead ECG parameters

2.4 STUDY ENDPOINTS

2.4.1 Pharmacokinetic (PK) Endpoints for Insulin Aspart (IA)

(1) Primary PK Endpoints for IA
  (i) C_{IAmax}, defined as the maximum serum IA concentration;
  (ii) $AUC_{IA(0\text{-}12h)}$, defined as area under the curve (AUC) of serum IA concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for IA
  (i) $AUC_{IA(0\text{-}\infty)}$ of Insulin Aspart,
  (ii) $AUC_{IA(0\text{-}2h)}$,
  (iii) t_{max} for IA (t_{IAmax}),
  (iv) Apparent clearance (CL/F),
  (v) Apparent volume of distribution (Vz/F), and
  (vi) Half-life ($t_{1/2}$).

2.4.2 Pharmacokinetic (PK) Endpoints for Human Insulin (HI)

(1) Primary PK Endpoints for HI
  (i) C_{HImax}, defined as the maximum serum HI concentration;
  (ii) $AUC_{HI(0\text{-}12h)}$, defined as area under the curve (AUC) of HI concentrations for time 0 to 12 hours; and

(2) Secondary PK Endpoints for HI: t_{max} for HI (t_{HImax})

2.4.3 Pharmacodynamic (PD) endpoints

The PD parameters are used to measure the effect of the study drug over time as measured by the hyperglycemic-euglycemic clamp procedure. During the clamp procedure, blood glucose concentrations are held at the target (=baseline – 5 mg/dL) after the administration of the study drugs by adjusting the exogenous glucose infusion rate (GIR in mg/kg/min). The GIR is defined as the infusion rate of intravenously administered glucose required to maintain the target blood glucose level. GIR data will be adjusted by the body weight.

(1) Primary PD Endpoints

- ***GIR_{max}***: Maximum GIR, defined as maximum infusion rate of glucose administered intravenously needed to maintain target blood glucose level.

- ***$AUC_{GIR(0\text{-}12h)}$***: defined as area under the curve (AUC) of GIR, i.e. the total amount of glucose infused over the duration of the clamp procedure.

(2) Secondary PD Endpoints

- $AUC_{GIR(0\text{-}last)}$,
- $AUC_{GIR(0\text{-}1h)}$,
- $AUC_{GIR(0\text{-}2h)}$,
- $AUC_{GIR(0\text{-}4h)}$,
- $AUC_{GIR(4\text{-}12h)}$,
- $tGIR_{max}$, time until maximum glucose infusion rate is reached,
- $tGIR_{onset}$, time of start of GIR post-dose,
- $tGIR_{last}$, time of last measurable GIR,
- GIR_{last}, value of last measurable GIR,
- $tGIR_{50\%E}$, defined as the time to 50% maximal GIR before $tGIR_{max}$,
- $tGIR_{50\%L}$, defined as the time to 50% maximal GIR after $tGIR_{max}$.

3. STUDY DESIGN AND OUTCOMES

3.1 STUDY DESIGN

This is a pivotal single dose, double-blinded, randomized, two-treatment, two-period, crossover, euglycemic glucose clamp study to assess the safety, pharmacokinetic (PK) and pharmacodynamic (PD) profiles of insulin I004 and the RP, NovoLog® in healthy subjects. Each period will include a 12-hour clamp and blood sampling procedure. The designed treatments in this study are summarized in **Table 1**.

Table 1. Treatments and Dose for I004-CL-B Study

Treatment	**Treatment-T**	**Treatment-R**
Study Drug Name	I004	Novolog
Manufacturer	[redacted]	[redacted]
API and Source	Insulin Aspart	Insulin Aspart
Dosage & Strength	Sterile Solution 100U/mL	Sterile Solution 100 U/mL
No. of Subjects	60 healthy volunteers participate in two (2) crossover treatment sequences; T -R or R - T	
Dose Regimen	0.2 U/kg	0.2 U/kg
Delivery Path	Subcutaneous Injection	Subcutaneous Injection

The study is conducted in healthy male and female adult subjects 18 to 65 years of age with a targeted total of sixty (60) evaluable subjects at the completion of the study.

The study includes one (1) screening visit, two (2) study visits (separated by at least 7-21 days) and a follow-up visit (within 1 to 14 days after the second study visit). All subjects will be screened for enrollment before being randomly assigned to one of the two treatment sequences, TR or RT. According to the randomized treatment sequence, at each study visit, a qualified subject will be treated with one of the two (2) treatments before undergoing a euglycemic clamp that lasts for 12 hours. At each study visit, twenty-eight (28) serial PK blood samples after treatment and during the clamp period (12 hours) will be collected for serum insulin aspart, C-peptide, and endogenous human insulin measurements. The GIR required to keep blood glucose at ±10% of target level (i.e. baseline – 5 mg/dL) will be recorded every one minute over 12 hours and data will be used to calculate PD parameters.

Basically, the Clamp controls the GIR based on ±10% of baseline minus 5 mg/dL.

Safety and tolerability will be assessed at screen visit and at each study visit. Safety and tolerability data, including adverse events will be documented. Participation in the study is expected to last up to 10 weeks. The two (2) study visits, and main study procedures, are outlined in the Summary of Activities below (**Table 2**):

Table 2. Summary of Activities in All Visits and EOS

ASSESSMENT	SCREEN	IN-HOUSE PERIOD 1	WASH-OUT	IN-HOUSE PERIOD 2	FOLLOW UP[1]
IP administration		X		X	
Informed consent	X				
Assess eligibility criteria	X				
Demography	X				
Medical history	X				
History of prior Insulin exposure/use	X				
Sequestered in clinic		X		X	
Physical examination (PE)	X				X
Hematology, serum chemistry & urinalysis[5]	X				X
HbA1c	X				
Coagulation	X				
TSH	X				
Hep B, Hep C, HIV	X				
FSH if postmenopausal	X				
Pregnancy test(serum)	X				
Pregnancy test(urine)		X		X	X
Urine drug screen & alcohol breath test	X	X		X	X
Weight	X	X		X	
Height, BMI	X				
Vital signs	X	X[2]		X[2]	X
12-lead ECG	X	X[3]		X[3]	X
Standardized meals		X		X	
Record concomitant medications	X	X		X	X
Record AEs	X	X		X	X
Assess check-in criteria		X		X	
Randomization		X			
Injection site assessment		X[4]		X[4]	
Clamp procedure		X		X	
PK/PD assessments		X		X	
Blood glucose (YSI)	X	X		X	

[1] To be performed after second dosing or at early termination
[2] On each Day 1, vital signs to be measured 15 (± 5) min pre-clamp/pre-dose and at 5 (± 3), 60 (± 10), 180 (± 20) and 720 (± 30) min post-dose.
[3] On each day 1, ECG 15 (± 5) min pre-clamp/pre-dose and at 5 (± 3) min, 60 (± 10) min, 180 (± 20) min and 720 (± 30) min post-dose.
[4] Assessment of reaction at 15 min, 60 min and 720 min post-dose.

3.2 OUTCOME MEASUREMENT

3.2.1 PK Measurements

At each of the two (2) study visits, PK blood samples will be collected from each subject at 28 scheduled time points (**Table 3**).

Table 3 PK Blood Sampling Schedule (~6 mL per sample)

Seq. #	Scheduling	Time window	Sample No.,"XX"	#	Scheduling	Time window	Sample No.,"XX"
1	-60 min	± 5 min	01	15	75 min	±3 min	15
2	-30 min		02	16	80 min	±5 min	16
3	0 min	±1 min	03	17	90 min		17
4	5 min		04	18	105 min		18
5	10 min		05	19	120 min		19
6	20 min	±2 min	06	20	150 min		20
7	25 min		07	21	180 min	±10 min	21
8	30 min		08	22	210 min		22
9	40 min	±3 min	09	23	4 h		23
10	50 min		10	24	5 h		24
11	55 min		11	25	6 h		25
12	60 min		12	26	8 h	±20 min	26
13	65 min		13	27	10 h		27
14	70 min		14	28	12 h		28
Total							28 (168mL)

PK samples are analyzed using an established and validated testing method in Amphastar R&D laboratories.

3.2.2 PD Measurements

At each study visit, the GIR required to maintain blood glucose at the target level (±10%) is recorded every minute for 12 hours and the data are used to calculate PD parameters.

3.2.3 Data for Safety Evaluation

The following safety parameters are monitored, documented and summarized:

a) Vital signs, i.e., body temperature, blood pressure (SBP/DBP), respiration rate and heart rate (HR), at:
   – Screening;
   – Pre-dose baseline, and 5 (±3) min, 60 (± 10) min, 180 (± 20) min and 720 (±30) min post-dose at both study visits.

b) 12-Lead ECG (Routine QT and QTc analysis) at:
   – Screening;
   – Pre-dose baseline, and 5 (±3) min, 60 (± 10) min, 180 (± 20) min and 720 (±30) minpost-dose at both study visits.

c) Results obtained from any clinically significant findings in physical examinations, CBC, metabolic panel and urinalysis (UA) for all subjects at:
   – Screening;
   – End of study evaluation.

d) Concomitant medication record.

3.2.4 Tolerability Assessments

After injection of the study drug, the injection site is marked with a pen. Assessment of study drug injection site is performed at 15 min, at 60 min and at 720 min after dosing. The local reaction from the injection site, the insertion site, and the adhesive is evaluated quantitatively using a Draize scale or similar scale by qualified study staff. If an injection site reaction like pain on palpation, itching, erythema, edema, induration is observed, it must be recorded as an AE and then is evaluated using the following scale:

Erythema is evaluated as follows:

- 0 – No erythema
- 1 – Very slight erythema (barely perceptible)
- 2 – Well-defined erythema
- 3 – Moderate to severe erythema
- 4 – Severe erythema (beet redness) to slight eschar formations (injuries in depth)

Edema is evaluated as follows:

- 0 – No edema
- 1 – Very slight edema (barely perceptible)

- 2 – Slight edema (edges of area well defined by definite raising)
- 3 – Moderate edema (raised approximately 1 mm)
- 4 – Severe edema (raised more than 1 mm and extending beyond the area of exposure)

The diameter of the affected area is measured in centimeters with a paper tape and the condition of the injection site is recorded. Digital photography will be used to document all positive injection site reactions. In case of clinically significant injection site reactions, subjects may undergo a dermatologic consultation.

3.2.5 Data for Adverse Drug Event Evaluation

All subjects will be queried for adverse drug events (ADEs) including injection site tolerability assessment, and ADEs must be recorded with all related information.

4. DATA POINTS OF PK AND PD

4.1 PK DATA: GENERAL CONSIDERATIONS

In this clinical study, 28 PK data points were collected for each treatment of each subject, and analyzed using a validated LC/MS analytical method. The PK data should be reported with reasonable number of significant figures, as rounded to 10% of the qualitative limit (QL) of the analytical method.

The following rules must be followed for the PK data points:

- Missed PK samples and the reasons must be tabulated;
- Un-analyzed PK samples and the reasons must be tabulated; and
- Re-assay of PK samples must be recorded. Both original data and re-assay data must be reported, which one should be used as the final data must be specified and the corresponding rationale should be reported and tabulated.

4.2 PK SAMPLES COLLECTED OUTSIDE THE PK SAMPLING WINDOW DEFINED BY THE PROTOCOL

(1) Basic Definitions Related to the PK Sampling Window

Being outside the pre-specified PK sampling window is a common protocol deviation. This type of protocol deviation can be flagged or excluded, as discussed below.

Assuming three (3) adjacent PK sampling time points are t_{j-1}, t_j, and t_{j+1} respectively, as demonstrated in **Figure 1** below.

The time gap of PK sampling time points t_j, from the left and right sampling points t_{j-1} and t_{j+1} can defined as follows by Eqs. (1) and (2), respectively.

$$g_j^- = t_j - t_{j-1} \quad (1)$$

$$g_j^+ = t_{j+1} - t_j \quad (2)$$

where, j = 1 to 25, and $t_1 = 0$, $t_{25} = 600$min (10hr).
For the last sampling point 720 min, $g^+ = g^- = 720 - 600 = 120$ (min).

(2) Criterion for Flag or Severe Protocol Deviation when Outside Sampling Window

In the case of a PK sample at time point j being collected outside the sampling window of $t_j \pm w_j$, a "flag-allowance" for the PK sample can be reasonably assigned to determine to flag or to exclude the corresponding PK sample, as follows:
The "flag allowance" for left (F^-) and right (F^+) sides, which are time intervals out of the specified sampling window, and are defined below, respectively:

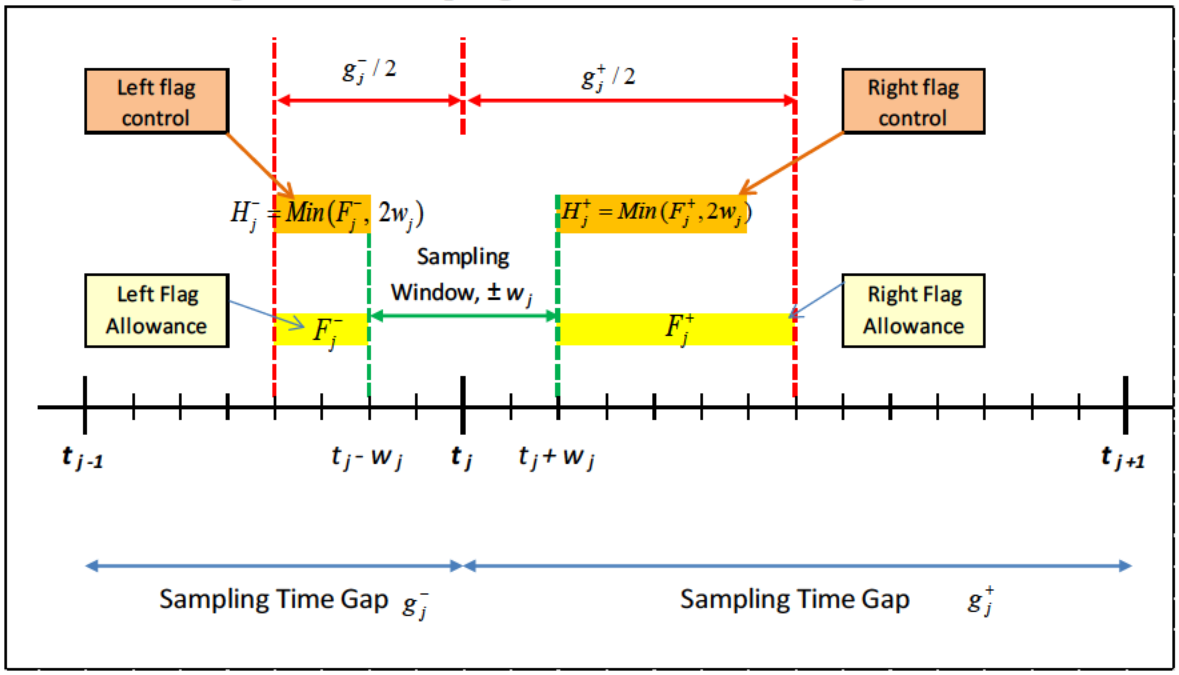
$$F_j^{\pm} = Max\left(\frac{1}{2} g_j^{\pm} - w_j, 0\right) \qquad (3)$$

The flag allowance $F^{\pm}$ is defined to avoid any overlapping between two adjacent PK sample timings. To be conservative, the final flag control, denoted as time intervals $H^{\pm}$, is defined as

$$H_j^{\pm} = Min\left(F_j^{\pm}, 2w_j\right) \qquad (4)$$

Namely, the flag control cannot be more than two (2) times of the specified window.

Figure 1. PK Sampling Points, Window and Flag Control



Therefore, the maximum allowable out-of-window time, denoted as $H_j^{\pm}$ can be obtained (see **Figure 1**). The proposed flag control of out-of-sampling-window for PK samples in this study are listed in **Table 4** below. Thus

- All PK samples outside the sampling window, but still within the final flag control are recorded as "flag" and "protocol deviation", but not "severe protocol deviation".

- All PK samples outside the final flag check are recorded as a "severe protocol deviation".

Table 4. Flag Control of Out-of-Sampling-Window for I004-B PK Samples

Sample Numbers	Sampling Plan					Time Range, min		Flag Allowance, min		Flag Control, min	
	tj, min	tj, hr	±	$w_{j,\ min}$		$g^-/2$	$g^+/2$	F^-	F^+	H^-	H^+
C1	0										
C2	5		±	1	min	2.5	2.5	1.5	1.5	1.5	1.5
C3	10		±	1	min	2.5	5	1.5	4	1.5	2
C4	20		±	2	min	5	2.5	3	0.5	3	0.5
C5	25		±	2	min	2.5	2.5	0.5	0.5	0.5	0.5
C6	30		±	2	min	2.5	5	0.5	3	0.5	3
C7	40		±	3	min	5	5	2	2	2	2
C8	50		±	3	min	5	2.5	2	0	2	0
C9	55		±	3	min	2.5	2.5	0	0	0	0
C10	60		±	3	min	2.5	2.5	0	0	0	0
C11	65		±	3	min	2.5	2.5	0	0	0	0
C12	70		±	3	min	2.5	2.5	0	0	0	0
C13	75		±	3	min	2.5	2.5	0	0	0	0
C14	80		±	5	min	2.5	5	0	0	0	0
C15	90	1.5	±	5	min	5	7.5	0	2.5	0	2.5
C16	105	1.75	±	5	min	7.5	7.5	2.5	2.5	2.5	2.5
C17	120	2	±	5	min	7.5	15	2.5	10	2.5	10
C18	150	2.5	±	5	min	15	15	10	10	10	10
C19	180	3	±	10	min	15	15	5	5	5	5
C20	210	3.5	±	10	min	15	15	5	5	5	5
C21	240	4	±	10	min	15	30	5	20	5	20
C22	300	5	±	10	min	30	30	20	20	20	20
C23	360	6	±	10	min	30	60	20	50	20	20
C24	480	8	±	20	min	60	60	40	40	40	40
C25	600	10	±	20	min	60	60	40	40	40	40
C26	720	12	±	20	min	60	60	40	40	40	40

4.3 QUALITY OF PD CLAMP DATA

At each study visit, the PD GIR required to maintain blood glucose at the target level (±10%) by clamp technology is recorded every minute for 12 hours. The target level is "baseline -5 mg/dL". The baseline is defined as the average of the available three (3) glucose levels at -30

min, -20 min and -10 min time measure by YSI 2300 STAT Glucose Analyzer for the subject at the treatment. If one or two of these three (3) glucose levels are missed. The data should be flagged.

4.3.1 Precision of Clamp Data

The precision is reported as CV (coefficient of variance) of device blood sugar. For a given subject *i* in a given treatment *X* (*X*=*T* or *R*) at a given time point *t*, the blood glucose (BG) level is denoted as $g_{i,X}(t)$, and the average of BG is obtained as

$$G_{i,X} = \frac{1}{n_t}\sum_t g_{i,X}(t) \qquad (5)$$

Calculations for CV by Eq. (5) is based on each clamp from the minute that the glucose infusion rate is initiated (Start of GIR[a]) to the minute that the glucose infusion rate is no longer required (End of GIR[b]) or at the end of the clamp at the 12-hour mark, whichever appears first. Note that an End of GIR is not necessarily an end of the clamp. In Eq. (5), n_t is total number of GIR data for the subject in the treatment per the above calculation method.

The precision for the clamp data point, denoted as $p_{i,x}$, can be measured using the coefficient of variance (CV, in %) as defined below:

$$p_{i,X} = \frac{\sigma_{i,X}}{G_{i,X}} \times 100\% \qquad (6)$$

where $\sigma_{i,X}$ is the standard deviation of the BG data of $g_{i,X}(t)$. It is expected that $p_{i,X} < 12\%$.

For the subjects with clamp precision ≥10%, the data should be flagged and tabulated. It $p_{i,X}$ ≥12%, the clamp data for the treatment of the subject should be rejected.

The clamp precision for a given treatment *X*, is then calculated as

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[a] **Start of GIR**: After dosing of Insulin Aspart, the onset of insulin action was identified by a decline of 5mg/dL or more for glucose concentration from the baseline level, the corresponding onset time is denoted as t_{onset}. At or after t_{onset}, the first time point with GIR(t)>0, is the start of GIR.

[b] **End of GIR**: If the last time point with GIR(t_{last}) > 0, then starting from t=t_{last}+1, the glucose infusion rate is no longer required, the time for end of GIR is Min(t_{last}+1, 720).

$$P_X = \frac{1}{n_X}\sum_{i=1}^{n_X} p_{i,X} \qquad (7)$$

where n_x is the number of subjects in the treatment X.

4.3.2 Deviation from Target (DFT)

The clinical protocol required that "the individual blood glucose clamp target level will be determined for each subject by the mean fasting glucose level minus 5 mg/dL.", which can be defined as g_0.

The baseline of blood glucose is the mean fasting glucose level from three (3) results measured by YSI 2300 STAT Glucose Analyzer at -30min, -20min, and -10min.

The actual blood glucose level for each corresponding clamp data point is denoted as $g_{i,X}(t)$. The average deviation from the target (DFT) for a given subject i in treatment X is then defined as

$$d_{i,X} = \frac{1}{n_t}\sum_{t} \frac{g_{i,X}(t) - g_0}{g_0} \times 100\% \qquad (8)$$

In calculation DFT (Eq. (9)), the time t runs the same as that for Eq. (5). It is also expected $d_{i,X}$ <12%. For the subjects with DFT ≥10%, the data should be flagged and tabulated. It $d_{i,X}$ ≥12%, the clamp data should be rejected.

The DFT for a treatment X is obtained by the following calculation:

$$D_X = \frac{1}{n_X}\sum_{i=1}^{n_X} d_{i,X} \qquad (9)$$

where n_x is the number of subjects in the treatment X.

4.3.3 Accuracy

Accuracy (denoted as A) is defined as mean difference of all paired BG measurements of device vs reference method represented as a percentage.

$$A = \frac{1}{n'_t} \sum_t \left(\frac{g_{i,X}(t) - g'_{i,X}(t)}{g'_{i,X}(t)} \times 100\% \right) \qquad (10)$$

where $g^{i,X}$ is the BG measured by Biostator,
$g'_{i,X}$ is the BG measured by YSI Glucose analyzer,
t is the time point when BG measurement by both methods are available, and t runs through all such both method data available time points;
n'_t is number of time points with BG measurement by both Biostator and YSI Glucose analyzer.

4.4 SMOOTHING OF PD DATA

During the 12-hour study, GIR PD data were collected every minute for each treatment (T or R) of each subject using clamp technique. In fact, 721 PD data points (0-720 min) were measured for each treatment (T or R) of each subject.

Due to the nature of the clamp technique, the PD data are highly fluctuating as a function of time, so smoothing is required before calculating PD parameters (such as GIR_{max}, AUC_{GIR}, etc.). [5] The smoothing procedure has been designed [6] to accommodate data for which

$$y_i = k(t_i) + \varepsilon_i \qquad (11)$$

where y_i is the actual PD observation as GIR at time i, k is a smooth function of PD, and the ε_i are random variables with mean 0 and constant scale, $\varepsilon_i \in \left(0, \sigma^2\right)$. Within such a framework, $\hat{y}_i$ is an estimate of $k(t_i)$. The assumption of smoothness allows points in a neighborhood of (t_i, y_i) to be used to form $\hat{y}_i$ [6].

For the actual smoothing, the SAS standard program Locally Estimated Scatterplot Smoothing (LOESS) in SAS/STAT 13.1 [7] is used.

The LOESS method uses weighted least squares to fit linear or quadratic functions of the predictors to the centers of the neighborhoods. The radius of each neighborhood is chosen so that the neighborhood contains a specified percentage of the data points. The fraction of the data, called the smoothing parameter, in each local neighborhood controls the smoothness of the estimated surface. Data points in a given local neighborhood are weighted by a smooth decreasing function of their distance from the center of the neighborhood [7]. Therefore,

$$M = s\,N \qquad (12)$$

where, N is the total number of experimental data points. For PD data of this study, N=721.

M is the number of data points in a given local neighborhood, and

s is the smoothing parameter, specifying a fraction of the total PD data.

This SAP suggests using smoothing parameter *s=0.0125,* so that M=9, namely there are 9 data points in each neighborhood.

5. CALCULATION OF RELATED STATISTICAL QUANTITIES

5.1 PK PARAMETERS

5.1.1 Primary Endpoints and Related Quantities

(1) ***C_{max}***, in units of pg/mL, is defined as the maximum of observed serum Insulin Aspart (IA) concentration for a given subject and given treatment over all PK sample concentrations:

$$C_{\max} = \max\left(C_1, \cdots, C_i, \cdots, C_n\right) \quad i = 1, 2, 3 \cdots n \tag{13}$$

where n=26, and C_i is rounded to 10% of the quantitative limit (QL) of the validated test method for IA.

(2) ***$AUC_{IA(0\text{-}12h)}$***, in units of pg/mL*hr, can be calculated per the trapezoidal rule and sampling time for each treatment of each subject as given the Eq. (14):

$$AUC_{0-12h} = \sum_{i=1}^{n-1} \frac{C_i + C_{i+1}}{2}\left(t_{i+1} - t_i\right) \tag{14}$$

where n is the number of available PK measurements,
t_1 is the baseline, and
t_n is the sampling time for the last scheduled PK measurement, t_n *=12 hours*.

5.1.2 Secondary Endpoints

(1) t_{max} is an observed value, corresponding when C_{max} appears, and is represented in units of "*minutes*", or "*min*".

(2) Partial ***$AUC_{IA(t_{m1}\text{-}t_{m2})}$***, in units of pg/mL*hr, can be calculated per the trapezoidal rule and sampling time for each treatment of each subject similarly as the Eq. (14):

$$AUC_{t_{m_1} - t_{m_2}} = \sum_{i=m_1}^{m_2-1} \frac{C_i + C_{i+1}}{2}\left(t_{i+1} - t_i\right) \tag{15}$$

where, t_{m1} and t_{m2} are the starting and ending sampling times of PK measurement for the partial ***$AUC_{IA(t_{m1}\text{-}t_{m2})}$***, respectively;

m is the number of available PK measurements.

The following partial AUC of Insulin Aspart will be calculated: (i) $AUC_{IA(0\text{-}1h)}$, (ii) $AUC_{IA(0\text{-}2h)}$, (iii) $AUC_{IA(0\text{-}4h)}$, and (iv) $AUC_{IA(4\text{-}12h)}$.

(3) ***$AUC_{IA(0\text{-}\infty)}$***, in units of pg/mL*hr, is defined as ***$AUC_{0\text{-}t^*}$***, with t* > t and meets

$$C(t^*) = C_1, \text{ if } C(t) > \max(C_1, QL) \quad (16)$$

where C_1 = baseline, and t=12 hrs, the time point for the last sample collection ;
QL is the analytical method's quantitation limit (QL)

When $C(t) \leq \max(C_1, QL)$,

$$AUC_{0-\infty} = AUC_{0-t} \quad (17)$$

Otherwise

$$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty} \quad (18)$$

$AUC_{0\text{-}t}$ can be calculated per the trapezoidal rule as given in the Eq. (14). $AUC_{t\text{-}\infty}$ can be obtained per the extrapolation method as given in the Eq. (19) below, based on

$$AUC_{t-\infty} = \int_{t_n}^{\infty} C(t)dt \approx -\frac{1}{K_e} Be^{-k_e t}\Big|_{t_n}^{\infty} = \frac{C_n - C_1}{K_e} \quad (19)$$

where $C_n = C(t_n)$ is the last available PK measurement for serum IA concentration;
K_e is the rate constant of elimination obtained per the method described in the next paragraph; and
B is a constant, irrelevant to time.

If the $AUC_{t\text{-}\infty}$ (Eq. (18)) cannot be found as a converged data for a subject, specifically:

- A positive K_e cannot be found; or
- The obtained $AUC_{t\text{-}\infty}$is much larger than 30% of $AUC_{0\text{-}t}$;

then, the data of $AUC_{0\text{-}\infty}$ for the treatment of the subject can be considered as not available.

(4) The Elimination Rate Constant, K_e, by Least Square Model

K_e will be obtained by the software output. In case the software cannot report the data of *Ke*, then *Ke* is calculated based on the least squares model from the logarithm of the last three (3)

or more (m=3, 4, 5, 6) available PK measurements of the treatment for the subject, per the following linear equation:

$$Ln\,[C(t)] = a + pt \qquad (20)$$

where p is the slope, and a is the intercept irrelevant to time, and K_e=-p.

A converged p_m can be accepted as the final p, for which the coefficient of variance (CV) of p_m and p_{m+1} is not more than 20%. When m is up to 6 and CV ≤ 20% is not achieved, the p_m with lowest CV with p_{m+1} will be used as the final p.

Here, m is the number of serum samples used to calculate K_e, where $K_e = -p$.

If there is no convergence of p or K_e that can be obtained, the corresponding data of $AUC_{0-\infty}$ for the subject is considered "not available".

K_e will not be included in tables or listings, but is referred to in the definitions of other PK parameters.

(5) Apparent clearance (CL/F), in units of L/hr, can be computed as:

$$CL/F = \frac{Dose}{AUC_{0-\infty}} \qquad (21)$$

(6) Apparent volume of distribution (Vz/F), in units of L, can be calculated as:

$$V_z/F = \frac{CL/F}{K_e} \qquad (22)$$

(7) Half-life ($t_{1/2}$) is defined as the time required for the drug concentration to decrease by a factor of one-half in terminal phase, and it can be estimated as:

$$t_{½} = \frac{\ln(2)}{K_e} \qquad (23)$$

Half-life is reported in units of hr.

5.2 PD ENDPOINTS

All PD parameters are obtained from the smoothed PD data reported as mg-glucose/kg/min.

5.2.1 Primary Endpoints

(1) ***GIR$_{max}$***, in units of mg-glucose/kg/min, is defined as the maximum of infusion rate of glucose administrated intravenously needed to maintain target blood glucose level:

$$GIR_{\max} = \max\left(GIR_1, \cdots, GIR_i, \cdots, GIR_n\right) \quad i = 1, 2, 3 \cdots n \tag{24}$$

where n=720 is the number of GIR recorded for a given treatment of a given subject.

(2) ***AUC$_{GIR(0\text{-}12h)}$***, in units of mg-glucose/kg, can be calculated per the trapezoidal rule as given the Eq. (25):

$$AUC_{GIR(0-12h)} = \sum_{i=1}^{n-1} \frac{GIR_i + GIR_{i+1}}{2}\left(t_{i+1} - t_i\right) \tag{25}$$

where n is the number of available PD measurements,
t_1 =0, and t_n = 720 minutes (*12 hours)*.

5.2.2 Secondary Endpoints

(1) *tGIR$_{max}$*, defined as time until maximum glucose infusion rate is reached is an observed value, corresponding when GIR$_{max}$ appears, and is represented in units of "*minutes*", or "*min*".

(2) Partial ***AUC$_{GIR(tv1\text{-}tv2)}$***, in units of mg/kg, can be calculated per the trapezoidal rule and sampling time for each treatment of each subject similarly as the Eq. (14):

$$AUC_{GIR\left(t_{v1} - t_{v2}\right)} = \sum_{j=v_1}^{v_2-1} \frac{GIR_i + GIR_{i+1}}{2}\left(t_{i+1} - t_i\right) \tag{26}$$

where, t_{v1} and t_{v2} are the starting and ending times of the partial ***AUC$_{GIR(tv1\text{-}tv2)}$***,, respectively;

The following partial AUC of GIR will be calculated:

- AUC$_{GIR(0\text{-}last)}$,

- $AUC_{GIR(0\text{-}1h)}$,
- $AUC_{GIR(0\text{-}2h)}$,
- $AUC_{GIR(0\text{-}4h)}$,
- $AUC_{GIR(4\text{-}12h)}$.

(3) $tGIR_{onset,}$ in units of "*minutes*", or "*min*", defined as time of start of GIR post-dose, is read as the earliest time from the smoothed PD curve when GIR>0.

(4) $tGIR_{last}$, in units of *min*, is read from the smoothed PD curve as the latest time when GIR>0.

(5) GIR_{last}, in units of %/*min*, defined as the value of last measurable GIR from smoothed PD curve.

(6) $tGIR_{50\%E}$, defined as the time reaching 50% maximal GIR before $tGIR_{max,}$ is obtained from the smoothed PD curve per interpolative rule.

$$tGIR_{50\%E} = \frac{\left(\frac{1}{2}GIR_{\max} - GIR_j\right)\left(t_j - t_{j+1}\right)}{GIR_j - GIR_{j+1}} - t_j \qquad (27)$$

where t_j and t_{j+1} are time point corresponding to GIR_j and GIR_{j+1}, the two adjacent data points on smoothed GIR-time curve;

$GIR_j \le \frac{1}{2}GIR_{\max}$ and $GIR_{j+1} > \frac{1}{2}GIR_{\max}$;

$t_j < tGIR_{\max}$.

(7) $tGIR_{50\%L}$, defined as the time reaching to 50% maximal GIR after $tGIR_{max,}$ is obtained from the smoothed PD curve per interpolative rule.

$$tGIR_{50\%L} = \frac{\left(\frac{1}{2}GIR_{\max} - GIR_j\right)\left(t_j - t_{j+1}\right)}{GIR_j - GIR_{j+1}} - t_j \qquad (28)$$

where $GIR_j \ge \frac{1}{2}GIR_{\max}$ and $GIR_{j+1} < \frac{1}{2}GIR_{\max}$, $t_j \ge tGIR_{\max}$.

5.2.3 Evaluation of PD Clamp Study for Net Insulin Aspart

In this clamp study, glucose infusion by controlled GIR neutralizes/offsets the effect of insulin to maintain euglycemia at the target level. However, both endogenous human insulin (HI) and exogenous insulin aspart (IA) are present in blood. To assess the contribution of net IA to the PD

profile, the PD GIR data should be adjusted by the IA fraction of total insulin (HI + IA). For a given subject *i* in Treatment *X* at time *t*, the IA fraction (denoted as *f*) is defined as follows:

$$f_{IA}^{i,X}(t) = \frac{C_{IA}^{i,X}(t)}{C_{IA}^{i,X}(t) + C_{HI}^{i,X}(t)} \quad (29)$$

where *t* is the given time point for which PK data of both IA and HI are analyzed, and *C* is the concentration of corresponding insulins.

The smoothed GIR data are adjusted to the GIR that corresponding to the net IA by the calculation below:

$$G_{IA}^{i,X}(t) = G_{Smooth}^{i,X}(t) \times f_{IA}^{i,X}(t) \quad (30)$$

If the denominator of Eq. (29) =0 for a time point *t*, then $f(t)=1$, i.e., the GIR is not adjusted for the time point. The unadjusted PD data points will be tabulated.

The obtained $G_{IA}(t)$ data are further used to calculate the G_{max} and $AUC_{0\text{-}12hr}$ for the GIR corresponding to the net IA per Section 5.2.1. and further assess the 90% CI for T vs. R.

5.3 Safety-Related Statistical Quantities

Safety assessment are performed based on the safety data collected directly from the eCRF or lab report.

6. STATISTICAL DESIGN AND ANALYSIS

6.1 STUDY POPULATION

6.1.1 Evaluable Subjects and Per Protocol Population (PPP)

PPP-PK: Per-Protocol Population for PK Analysis

The PPP-PK is defined as all subjects who have received both study drugs during the study and are evaluable for both treatments. Primary PK analyses will be performed based on the PPP-PK.

In order to be evaluable for the primary analyses of this study, a subject must meet all of the following six (6) criteria for both treatments:

(1) Correct dose and administration for both T and R treatments.
(2) Nineteen (19) or more of 25 post-dose PK data (>75%) for serum concentration points are available;
(3) There are no more than four (4) consecutive missing PK data points;
(4) The baseline PK data point and the 12-hour post-dose PK data are available;
(5) At least five (5) of the seven (7) PK data points at 50, 55, 60, 65, 70, 75, and 80, post-dose are available;
(6) At least three (3) of the PK data points at 5, 6, 8, and 10 hours post-dose are available.

PPP-PD: Per-Protocol Population for PD Analysis

The PD BE evaluation will be performed based on the PPP-PD. An evaluable subject for PD primary endpoint analyses of this study must meet all of the following four (4) criteria for both treatment arms:

(1) Correct dose and administration for both T and R treatment;
(2) More than 90% per minute GIR records are available;
(3) The clamp precision < 12% for both T and R treatments; and
(4) The clamp Deviation From Target (DFT) <12% for both T and R treatments.

6.1.2 Treated Population (TP)

The "treated" population is defined as all subjects who have received any amount of study drug treatment.

Safety assessment are performed using the treated population.

The treated population will also be analyzed for the PK parameters, as supportive evidence to the PK profiles.

6.1.3 The "Intent-To-Treat" Population (ITT)

The ITT population is defined as all subjects who have been randomized and are treated or untreated with the study treatment.

6.1.4 Analysis with Randomization Code Deviation

Analysis of ITT and TP are not BE determination analyses. In cases where a subject's actual treatment has deviated from the randomized treatment assignment (which has been confirmed by site investigator(s) and Amphastar quality assurance), analysis of TP will follow the actual treatment received. Conversely, analysis of the ITT will follow the original randomization assignment[1].

However, the analysis for PPP, which is the determination analysis for BE, will exclude the subject with a deviation from the randomization code as specified in Section 5.1.1.

6.2 MISSING DATA HANDLING

6.2.1 Missing Data Handling for Evaluable Subjects

For an evaluable subject that was defined in Section 6.1.1, only a small portion of PK measurements, if any, are allowed to be missed, and the interpolation method will be used for imputation.

If the PK data at t=12 hrs is missed, the exploration method is allowed.

6.2.2 Missing Data Handling for Non-Evaluable Subjects

Non-Evaluable Subjects will be excluded from primary and secondary analysis.

6.3 DATA SETS

The data sets are defined based on the following two factors: (i) laboratory analysis and (ii) severe protocol deviation (SPD) assessment by the Management Review Board (MRB), constituted by the Quality Assurance (QA) department, Clinical Operations, the Clinical Science Group, and Regulatory Affairs.

6.3.1 The PK Datasets

The PK data based on PK sample analysis will have two sets of results:

- Final Reported Data (FRD): the results finally reported by the laboratory for PK analysis, which will include results from re-assayed samples due to various reasons, such as (i) out of calibration range, (ii) correction of laboratory error, etc.
- Initially Tested Data (ITD): the results reported by the laboratory for PK analysis, which will include only initial test results data, without any re-assay. It is acknowledged that ITD may have some incorrect data.

6.3.2 Severe Protocol Deviation Assessed by Management Review Board

The Management Review Board (MRB) may determine that some data should be excluded from PPP analysis due to severe protocol deviation (SPD). SPD include, but are not limited to,

- Full dose of study drug not administered (ICH category III);
- Use of prohibited drugs (ICH category IV);
- Violating drug, alcohol, or caffeine restriction;
- Sample handling (extreme deviations of sample isolation time, centrifuge time, sampling record etc.); and
- Severe out of the sampling window time (see **Section 5.6.3**).

The data for excluded samples due to SPD will be considered missing data (see **Section 5.2**).

Thus, two datasets will exist for

- SPD-excluded (SPD-E) dataset: identified SPD data are excluded and are considered as missing data;
- SPD-included (SPD-I) dataset: identified all SPD data are still used in the data analysis.

6.3.3 Datasets used for Statistical Analysis

Combining the above two factors, in total there are four (4) datasets for PK and PD evaluation as summarized in **Table 5**, and **Table 6**, respectively below:

Table 5. Datasets for I004-B: PK Evaluation

Factors to Determine Datasets			Laboratory PK Data Analysis	
			Initially-Tested Data (ITD)	Final Reported Data (FRD)
Assessment for Severe Protocol Deviation (SPD)	No SPD Observed	-	ITD	FRD
	SPD Observed	SPD-excluded (SPD-E)	ITD + SPD-E	FRD + SPD-E
		SPD-included (SPD-I)	ITD + SPD-I	FRD + SPD-I

The PK BE statistical analysis will be conducted for all four (4) datasets (**Table 5**). Among the datasets, the final reported data for SPD-excluded (FRD + SPD-E) dataset is the most reasonable one and should be considered as the formal results for PK evaluation.

Table 6 Datasets for I004-B: PD Evaluation

Factors to Determine Datasets			Final Reported Data for PD (FRD)
Assessment for Severe Protocol Deviation (SPD)	No SPD Observed	-	FRD
	SPD observed	SPD-excluded (SPD-E)	SPD-E=FRD
		SPD-included (SPD-I)	SPD-I

The PD BE statistical analysis will be performed for all two (2) datasets (**Table 6).** Among the datasets, the final reported data for the SPD-excluded (FRD + SPD-E) dataset is the most reasonable one and should be considered as the formal results for the PD evaluation.

6.4 STATISTICAL ANALYSIS

6.4.1 Demographic Data

Demographic data and baseline characteristics are reported for each subject. For all subjects as one group, continuous variables will be summarized with n, mean, and standard deviation, while frequency counts and percentages of subjects within each category will be provided for categorical data.

6.4.2 Endpoints Evaluation

6.4.2.1 Primary Analysis

The objective of this study is to assess and compare the PK/PD profile of I004 and Novolog.

The geometric mean ratio and confidence intervals of the primary analyses should meet FDA bioequivalence requirements: the 90% confidence interval is within 80.00 – 125.00%.

The two (2) null hypotheses for the standard two one-sided tests procedure are :

$$H_{01}: \quad \mu_T - \mu_R \leq \theta_1 \tag{31}$$

$$H_{02}: \quad \mu_T - \mu_R \geq \theta_2 \tag{32}$$

and the alternative hypothesis is:

$$H_a: \quad \theta_1 < \mu_T - \mu_R < \theta_2 \tag{33}$$

where, for PK dataset, μ_T and μ_R are the natural logarithms of geometric means of $\boldsymbol{C_{max}}$ and $\boldsymbol{AUC_{IA(0\text{-}12h)}}$ for serum IA concentration of Treatment T and Treatment R, respectively; for PD dataset, μ_T and μ_R are the natural logarithms of geometric means of $\boldsymbol{GIR_{max}}$ and $\boldsymbol{AUC_{GIR(0\text{-}12h)}}$ for smoothed GIR curve of Treatment T and Treatment R, respectively.

The parameters θ_1 and θ_2 are equal to –0.223 (i.e., ln 0.8) and 0.223 (i.e., ln 1.25), respectively.

PK/PD endpoints will be analyzed using ANOVA methods with the effects of Treatment groups, Period, Sequence and Patient-within-Sequence. The following model is used:

$$Y_{ijkh} = \mu + T_j + Q_k + P_h + S_{i,k} + e_{ijkh} \quad (34)$$

where μ is the overall mean of the natural logarithms of $\boldsymbol{C_{max}}$, $\boldsymbol{AUC_{0\text{-}12hr}}$ and $\boldsymbol{GIR_{max}}$, $\boldsymbol{AUC}_{GIR(0\text{-}12hr)}$ denotes the effect of the jth treatment, Q_k denotes the effects of kth sequence, P_h denotes the effect of the hth period, $S_{i,k}$ denotes the effect of ith subject nested within kth sequence, and e_{ijkh} is independent random errors in observing Y_{ijkh}. For 2x2 crossover design as this study, random errors e_{ijkh} follow the distribution $N(0, \sigma)$, where the population standard deviation σ can be approximated as:

$$\sigma \approx s = \sqrt{MSE} = \sqrt{\frac{SSE}{n_{AB} + n_{BA} - 2}}. \quad (35)$$

The mean square error (MSE) and the sum of square error (SSE) will be obtained directly from the SAS output.

The 90% confidence intervals (CI) will be computed as follows:

$$90\%CI = \Delta \pm t_{1-\alpha/2,df} \times \sigma \quad (36)$$

where α=10%, and Δ is the difference of least square means of the logarithms of PK/PD endpoints for treatments T and R.

After being transformed back from the logarithm form, the result per Eq. (27) becomes the 90% confidence intervals of the geometric mean of the ratio of PK/PD parameters (T/R).

6.4.2.2 Secondary Analysis

Similar analysis also will be performed on secondary PK/PD endpoints between Treatment T and R.

6.4.3 Safety Assessment

6.4.3.1 Vital Signs and ECG Data

The mean, standard deviation and range will be calculated for all vital signs and ECG data for each treatment.

6.4.3.2 Laboratory Test Data

The results of laboratory tests, including CBC, metabolic panel and UA, will be assessed and comparisons between data at Screening and that at the EOS. If there are any significant changes, further investigation and evaluation are performed.

6.4.3.3 Adverse Drug Events (ADE)

Safety will be assessed by tabulation of ADE and will be presented with descriptive statistics for each treatment arm. Adverse events will be summarized by treatment group. Treatment groups will be compared with respect to the incidence of each type of adverse event observed.

Adverse events will be classified per symptoms and system organ class on the basis of MedDRA Preferred Terminology (PT), and summarized for each treatment arm. Severe or Serious ADEs will be summarized and listed.

All information pertaining to adverse events noted during the study will be listed for subject, ADE code, onset time and date, phase, severity, action taken, relationship to study drug, subject outcome, time resolved and date, seriousness and causing withdrawal or not.

6.4.3.4 Early Termination for Safety Reasons

Any early termination are listed with primary reasons per treatment arm as part of the safety assessment. Any early termination due to safety reasons must be specifically listed.

6.5 INTERIM ANALYSES

No interim analyses are planned to be performed for this study.

6.6 DATA QUALITY ASSURANCE

6.6.1 Data Input

Study data is entered into eCRF (Electronic Case Report Form) by the site personnel. The eCRF data management system is a web based application that is used to manage study data. The eCRF data management system is validated to comply with FDA 21 CFR Part 11.

6.6.2 Data Quality Assurance and Monitoring

The trial will be monitored according to current Amphastar Standard Operating Procedures (SOPs).

The sponsor site monitors will monitor investigational activities for the purpose of subject safety, study compliance to applicable regulatory guidelines, and study source data for quality and integrity.

The review of the eCRF study data entries is also conducted by the sponsor site monitors to ensure that eCRF data is accurate and traceable to the reliable study source. The Investigators will permit Amphastar authorized monitors to access the subject source documents, clinical supplies dispensing and storage area and study documentation as frequently as necessary and agrees to assist the site monitors with their activities. In addition, the eCRF data is also reviewed remotely by authorized personnel (Data Manager and safety Monitors) for data completeness and format. The Investigator will review the eCRF; provide missing or corrected data and e-sign the eCRF at the close out of the study. Personal or subject identifying information will be treated as confidential and will NOT be publicly accessible. The trial information may be reviewed by regulatory authorities or independent QA auditors. The study site may be inspected during or after completion of the study. The Investigators agree to allow inspectors from regulatory agencies to have access to all study records, including subject source documents and eCRF study data. By participating in this study, the Investigators agree to these requirements and will assist the inspectors in their duties.

6.6.3 Database and Computer Programs for Statistical Analysis

A specialized trial-specific database is designed to capture data in such a way that it facilitates reporting and analysis, i.e. minimal data manipulation and programming to complete the analysis.

The data collection and reporting system is fully tested and validated. The system is demonstrated to be accurate, reproducible and secure.

The computer programs used for statistical analysis are validated.

7. REFERENCES

[1] Comparison of the Pharmacokinetics (PK) and Pharmacodynamics (PD) similarity of Proposed Biosimilar Rapid-Acting Insulin Aspart (I004) and NovoLog® after Single-dose Subcutaneous Administration to Healthy Volunteers, Protocol No.: API-I004-CL-B, Ver. 1.3, (05/15/2020)

[2] FDA Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection" dated February 2019

[3] Peter Armitage and Theodore Colton, Encyclopedia of Biostatistics, John Wiley & Sons, New York, (1999) p705.

[4] Regarding clamp method: FDA CDER, Review document 205692Orig1s000.

[5] Tim Heise, K. Ka[lan, and H.L. Haahr, J. Diabetes Sci Technol , 12(2) 356-363, 2018.

[6] W. S. Cleveland, J Am. Statistical Assoc. 829-8336, Dec. 1979,

[7] SAS/STAT® 13.1.

Attachment:

Template of Statistical Analysis Output for Clinical Study Protocol No. API-I004-CL-B (Available Upon Request).