CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-810

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

ADDENDUM to CLINICAL PHARMACOLOGY REVIEW

NDA:21-809; 21-810	Submission Date: 03-02-2007
Brand Name	NovoLog® Mix 30/70; NovoLog® Mix 50/50
Generic Name	30% Insulin aspart protamine suspension and 70% insulin aspart injection, [rDNA origin] 50% Insulin aspart protamine suspension and 50% insulin aspart injection, [rDNA origin]
Reviewer	Xiaoxiong (Jim) Wei, M.D., Ph.D.
Team Leader (Acting)	Sally Y. Choe, Ph.D.
OCP Division	Division of Clinical Pharmacology 2
OND division	Division of Metabolism and Endocrinology Products
Sponsor	Novo Nordisk
Relevant IND(s)	65,182; ——— b(4)
Formulation; Strength(s)	Injection solution, 3 mg/mL
Dosing regimen	The dosage is adjusted for individual patients
Indication	Diabetes mellitus
Submission type	Major amendment to the submission for a complete response to NA Letter

Note of abbreviations:
IAsp: Insulin aspart
BIAsp: biphasic insulin aspart
BIAsp 30: biphasic insulin aspart 30% soluble/70% protracted
BIAsp 50: biphasic insulin aspart 50% soluble/50% protracted
BiAsp 70: biphasic insulin aspart 70% soluble/30% protracted

Table of Contents

1 Executive Summary

1.1 Recommendation

The Office of Clinical Pharmacology /Division of Clinical Pharmacology 2 (OCP/DCP2) has reviewed Trial 1746 submitted on March 2, 2007 as a major amendment to the complete response to the Agency's not approvable (NA) letter for NDA 21-809 for NovoLog Mix 30/70 (BIAsp 70) and NDA21-810 for NovoLog Mix 50/50 (BIAsp 50).

Based on the pharmacokinetic (PK) and pharmacodynamics (PD) analyses, BIAsp 70 and BIAsp 50 have been determined not to be distinct drug products from each other. However, as to BIAsp 70 and BIAsp 50 versus BIAsp 30, The Office of Clinical Pharmacology could not conclude whether the moderate differences (15-18% difference) observed in PD endpoint ($AUC_{GIR\ 4\text{-}12\ hrs}$) and 5 – 9% difference in PK endpoint ($AUC_{4\text{-}12\ hrs}$) will translate into clinically relevant benefits as distinct drug products in the treatment of diabetic patients. In addition, bioavailability was reduced by 24% for BIAsp 50 as compared to IAsp, the short-acting insulin product. b(4)

This recommendation should be conveyed to the sponsor as appropriate.

1.2 Summary of Clinical Pharmacology and Biopharmaceutics Findings

Trial 1746 showed that the two NovoLog mixes, BIAsp 70 and BIAsp 50, are not distinguishable from each other from PK and PD analyses. Although the mean difference in $AUC_{GIR\ 0\text{-}2\ hrs}$ as the early phase PD endpoint was 14% between BIAsp 70 and BIAsp 50, statistical analysis indicates that it is not statistically significant. The difference in the intermediary phase PD endpoint, $AUC_{GIR\ 4\text{-}12\ hrs}$, was 4%. While the mean difference in early phase PK endpoint of $AUC_{IAsp\ 0\text{-}2\ hrs}$ was reported to be 48%, the mean difference in the intermediary PK endpoint of $AUC_{IAsp\ 4\text{-}12\ hours}$ was only 4%.

Bigger differences were observed when BIAsp 70 and BIAsp 50 were compared to BIAsp 30. The difference in the PD endpoint of $AUC_{GIR\ 4\text{-}12\ hrs}$ was 15% as between BIAsp 70 and BIAsp 30, and 18% as between BIAsp 50 and BIAsp 30. Based on the composition of short- and long-acting insulins, the mean difference between BIAsp 70 and BIAsp 30 in PD endpoint should have been greater than that between BIAsp 50 and BIAsp 30. However, the result indicated the opposite (15% vs 18%), suggesting great variations in the study. The PK analysis showed that the differences in the intermediary phase parameter of $AUC_{IAsp\ 4\text{-}12\ hours}$ are minimal for all three NovoLog mixes. The differences in $AUC_{IAsp\ 4\text{-}12\ hours}$ were 6% as between BIAsp 70 and BIAsp 50, 9% as between BIAsp 50 and BIAsp 30, and 5% as between BIAsp 70 and BIAsp 30. Similar to the findings in the previous pivotal PK/PD Study 1086 of the original submissions, bioavailability is decreased with increasing fraction of long acting components in NovoLog mixes. Bioavailability was reduced by 30% for BIAsp 30 and by 24% for BIAsp 50, each as compared to IAsp, the short-acting insulin product.

2. Background

On March 2, 2007, the sponsor submitted a new clinical trial (Trial ID:BIAsp-1746) to the Agency, which extended the review cycle for additional 3 months for the submission dated August 30, 2006 as a complete response to the Agency's NA letter. In the original submissions for both NovoLog Mix 30/70 and NovoLog Mix 50/50, the Agency issued a not approvable (NA) letter for NDA 21-809 and NDA 21-810, respectively because [b(4)]

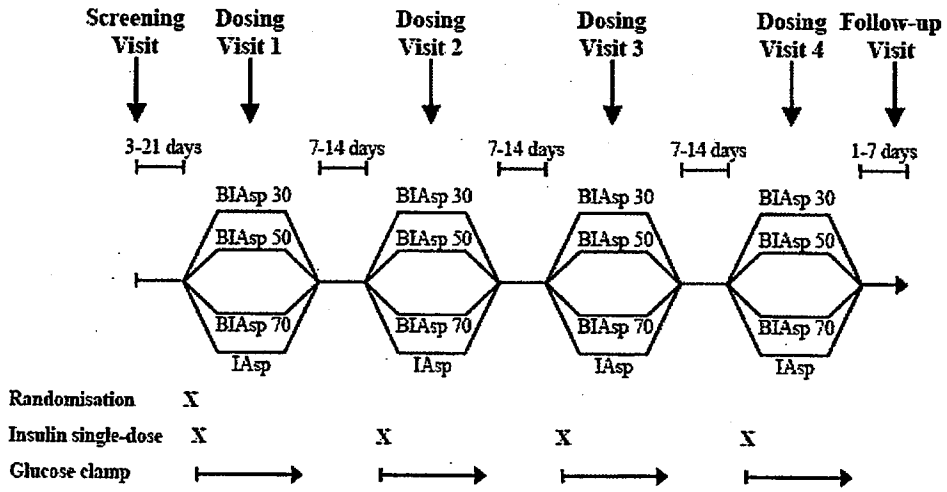
3. Trial BIAsp 1746

This is a double-blind, randomized, four-period crossover trial comparing the PK and PD after single dose of BIAsp 30, BIAsp 50, BIAsp 70 and insulin aspart in subjects with type 1 diabetes. The rationale sponsor described for the previous BIAsp-1086 trial showing subtle differences in PD between the different BIAsp-formulations was that the study was conducted in healthy subjects instead of patients. In particular, duration of action appears to be longer in healthy subjects compared to subjects with type 1 diabetes. Since the plasma glucose level decreases during the artificial, prolonged fasted state, maintenance of euglycemia may stimulate endogenous insulin secretion in healthy subjects and necessitate an exaggerated rate of glucose infusion. Therefore, in order to avoid any interference from endogenous insulin, the Trial 1746 was conducted in C-peptide negative patients with type 1 diabetes. The trial was conducted in the laboratory of Tim Heise, MD, Profil Institute for Metabolic Research, Ltd, Neuss, Germany, where trials for the Study 1806 and [redacted] were conducted. b(4)

The primary objective was to compare the early pharmacodynamics of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp based on GIR results at the dose of 0.4 U/kg. The secondary objectives were (1) to compare the pharmacokinetics of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp; (2) to compare other single dose PD properties as defined as standard GIR endpoints from the GIR curves among BIAsp 30, BIAsp 50, BIAsp 70 and IAsp; (3) to compare end of action as derived from the blood glucose concentrations during the clamp (end of action is defined as the time-point when blood glucose exceeds 160 mg/dL with no glucose infusion in the last 30 min or longer); (4) to compare PD properties derived from the serum NEFA concentrations among BIAsp 30, BIAsp 50, BIAsp 70 and IAsp and (5) to evaluate the safety of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp.

The study design and flow are schematically shown in Figure 1.

Figure 1. Trial design



Forty-six (46) subjects were screened. Thirty-two (32, twenty-one male and eleven female subjects) aged 21 to 52 years, with type 1 diabetes ranging 5 to 33 years were recruited and completed the trial. The mean BMI was 25 kg/m^2 (range 20 to 31 kg/m^2), mean HbA1c was 7.6% (range 6.3 to 8.5%), and C-peptide was ≤ 0.14 nmol/L in all subjects. All subjects were receiving basal-bolus treatment with soluble human insulin, insulin lispro, insulin glulisine, neutral protamine hagedorn insulin, insulin detemir or insulin glargine, but they were not previously treated with insulin aspart.

The results are summarized below.

Pharmacodynamic analysis:

The all four preparations for PD endpoints are presented in **Figure 1**. The values for the primary endpoint, $AUC_{GIR,0\text{-}2h}$, were derived from the GIR profiles and are summarized in **Table 1**. IAsp and the three BIAsp preparations were compared with respect to $AUC_{GIR,0\text{-}2h}$ using an ANOVA model. For $AUC_{GIR,0\text{-}2h}$, significant differences were demonstrated between IAsp and BIAsp 70 and between BIAsp 50 and BIAsp 30. $AUC_{GIR,0\text{-}2h}$ for IAsp was 26% higher than for BIAsp 70 and $AUC_{GIR,0\text{-}2h}$ for BIAsp 50 was 52% higher than for BIAsp 30. $AUC_{GIR,0\text{-}2h}$ for BIAsp 70 was 14% higher than for BIAsp 50 but this difference was not significant (p=0.0933) (**Table 2**).

Figure 2. Mean Smoothed Glucose Infusion Rate Profiles

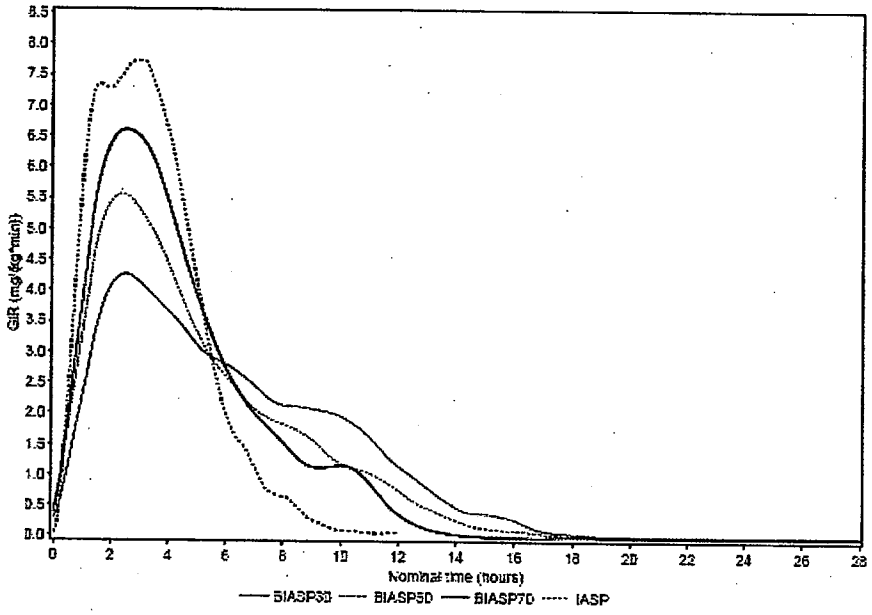


Table 1. Area under the GIR curve during the first 2 hours after s.c. injection of trial products ($AUC_{GIR,\ 0\text{-}2\ hr}$) in trial 1746 (from Table 14.2-8)

	IAsp	BIAsp 70	BIAsp 50	BIAsp 30
N	31	31	31	31
Mean (SD)	540	429	376	247

Table 2. ANOVA Comparison of ($AUC_{GIR,\ 0\text{-}2\ hr}$)

	IAsp vs BIAsp 70	BIAsp 70 vs BIAsp 50	BIAsp 50 vs BIAsp 30	BIAsp 70 vs BIAsp 30
Mean ratio	1.26	1.14	1.52	1.74
95% CI	1.08; 1.47	0.98; 1.33	1.31; 1.78	1.49; 2.03
P value	0.0038	0.0933	<0.001	<0.001

The secondary PD endpoints characterizing the time course of GIR following trial drug administration were derived from the GIR profiles and are summarized in **Table 3.**

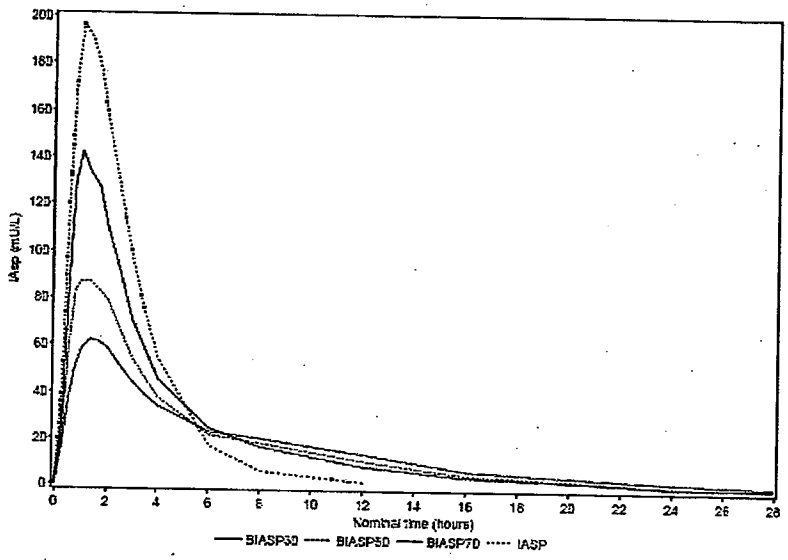
Table 3. Summary of analysis of secondary GIR endpoints in Trail 1746 (derived from Table 14.2.9)

Parameter	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIasp 70	BIAsp 70 / BIasp 50	BIAsp 50 / BIasp 30	BIAsp 70 / BIAsp 30
$AUC_{GIR,\ 0\text{-}4\ hr}$ (mg/kg)								
N	31	31	31	31				
mean	1416	1174	1001	725				
ratio					1.21	1.17	1.38	1.62
$AUC_{GIR,\ 4\text{-}12\ hr}$ (mg/kg)								
N	31	31	31	31				
mean	543	876	846	1029				
ratio					0.62	1.04	0.82	0.85
$AUC_{GIR,\ 0\text{-}6\ hr}$ (mg/kg)								
N	31	31	31	31				
mean	1901	1652	1396	1094				
ratio					1.15	1.18	1.28	1.51
$AUC_{GIR,\ 0\text{-}12\ hr}$ (mg/kg)								
N	31	31	31	31				
mean	2061	2124	1909	1777				
ratio					0.97	1.11	1.07	1.19
$AUC_{GIR,\ 0\text{-}28\ hr}$ (mg/kg)								
N	31	31	31	31				
mean	2060	2141	1972	1914				
ratio					0.96	1.09	1.03	1.12
$AUC_{GIR,\ 6\text{-}12\ hr}$ (mg/kg)								
N	29	31	30	31				
mean	51	356	497	661				
ratio					0.14	0.72	0.75	0.54
$AUC_{GIR,\ 6\text{-}28\ hr}$ (mg/kg)								
N	29	31	30	31				
mean	51	367	544	786				
ratio					0.14	0.67	0.69	0.47
GIR max (mg/kg/min)								
N	31	31	31	31				
mean	8.6	6.8	5.8	4.5				
ratio					1.27	1.18	1.29	1.52

Pharmacokinetic analysis:

The pharmacokinetic profiles of all four preparations are presented in Figure 3.

Figure 3. Mean Serum Insulin Aspart Profiles



The maximum concentrations (Cmax) of serum IAsp are summarized in **Table 4.** IAsp and the three BIAsp preparations were compared with respect to Cmax using the same ANOVA model as for the primary endpoint (see **Table 5**). For Cmax, significant differences were demonstrated among all four trial products. Cmax for IAsp was 35% higher than for BIAsp 70, Cmax for BIAsp 70 was 51% higher than for BIAsp 50 and Cmax for BIAsp 50 was 49% higher than for BIAsp 30. The other PK parameters over time profiles are summarized in **Table 6.**

Table 4. Cmax for serum IAsp and BIAsp preparations (mU/L)

	IAsp	BIAsp 70	BIAsp 50	BIAsp 30
N	31	31	31	31
Mean	191	141	94	63

Table 5. ANOVA Comparison on Cmax

	IAsp / BIAsp 70	BIAsp 70 / BIAsp 50	BIAsp 50 / BIAsp 30	BIAsp 70 / BIAsp 30
Mean ratio	1.35	1.51	1.49	2.25
95% C.I.	1.22; 1.50	1.36; 1.67	1.34; 1.65	2.03; 2.49
P value	<0.001	<0.001	<0.001	<0.001

Table 6. Summary of PK profiles in Trail 1746

Parameter	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIasp 70	BIAsp 70 / BIasp 50	BIAsp 50 / BIasp 30	BIAsp 70 / BIAsp 30
AUC IAsp, 0-2 hr (mU*h/L)								
N	31	31	31	31				
mean	261	192	130	87				
ratio					1.36	1.48	1.48	2.20
AUC IAsp, 0-4 hr (mU*h/L)								
N	31	31	31	31				
mean	460	337	239	172				
ratio					1.36	1.41	1.39	1.96
AUC IAsp, 4-12 hr (mU*h/L)								
N	31	31	31	31				
mean	90	142	136	150				
ratio					0.63	1.04	0.91	0.95
AUC IAsp, 0-6 hr (mU*h/L)								
N	31	31	31	31				
mean	530	407	294	224				
ratio					1.30	1.38	1.32	1.82
AUC IAsp, 0-12 hr (mU*h/L)								
N	31	31	31	31				
mean	570	490	383	326				
ratio					1.16	1.28	1.18	1.51
AUC IAsp, 0-28 hr (mU*h/L)								
N	31	31	31	31				
mean	576	536	438	401				
ratio					1.07	1.23	1.09	1.34
AUC IAsp, 0-inf (mU*h/L)								
N	29	31	30	31				
mean	589	564	474	442				
ratio					1.04	1.19	1.07	1.28
AUC IAsp, 6-28 hr (mU*h/L)								
N	31	31	31	31				
mean	32	116	130	170				
ratio					0.28	0.90	0.76	0.68
t1/2 (h)								
N	31	31	31	31				
Mean	2.2 (3.4)	4.2 (2.2)	5.5(6.5)	7.6 (10)				

Bioavailability:

The phenomenon of reduced bioavailability in Trial 1746 is similar to Trial 1086 though BIAsp 30 in type 1 diabetic patients exhibited a less magnitude in reduction of bioavailability in comparison with BIAsp 30 in healthy subjects (**Table 7**).

Table 7. The bioavailability with different NovoLog Mixtures in type 1 diabetic patients in Trial 1746 and in healthy subjects in Trial 1086

NovoLog Mix	Trial			
	1746		1086	
	AUC_{IAsp}, 0-28 hr (mU.mim./L)	% of Soluble IAsp	AUC_{IAsp}, 0-24 hr (mU.mim./L)	% of Soluble IAsp
Soluble IAsp	34560	100	18407 (3153)	100
BIAsp 70	32160	93	17628 (3121)	95.8
BIAsp 50	26280	76	13612 (2919)	74
BIAsp 30	24060	70	11486 (4254)	62.4
Neutral protamine IAsp	Not available	Not available	Not available	Not available

Analytical:

Insulin aspart was determined in serum by an enzyme linked immunosorbent assay (ELISA). The lower limit of quantification for this assay was 12.5 pmol/L and the detection limit is 5.3 pmol/L. Measurements were performed in the calibration range between 0 and 877 pmol/L after appropriate dilution of the samples. Quality control samples at three different levels were assayed in each analytical run (duplicate run). The concentration levels were 33.1, 378, and 668 pmol/L. The inter-assay precision (coefficient of variation) of the quality control samples ranged between ——)%. The mean inaccuracies of the quality control samples ranged between —— }%. b(4)

DSI Inspection:

The Division of Scientific Investigations (DSI) audited the clinical-pharmacodynamic and analytical-pharmacokinetic portions of the study 1746. The conclusions are as follows:

a. Dosing of subjects cannot be assured to have followed the randomization code.

b. For the experiments in the table below, the OCP/DMEP reviewer should evaluate the reliability of the GIRs between 0 to 120 min ($AUC_{GIR,0\text{-}120\ min}$), the primary endpoint

Subject ID Visit

Subject ID	Visit
#13	2
#35, #43	3
#32, #33	4
#25, #28	5

c. The nonesterified free fatty acids (NEFA) concentration data are unreliable as the NEFA assay was found to be deficient.

REVIEWER'S COMMENTS:

The sponsor chose only the early phase PD parameter as their primary endpoint. This reviewer thinks that both the early and intermediary phases of PK and PD profiles are important endpoints to characterize the premixes as distinct products from each other.

The exposures of insulin aspart mixes are much greater in type 1 diabetic patients in Trial 1746 compared to the Trail 1086 conducted in healthy subjects (see Table 7). Part of reasons for a greater exposure is due to higher dose, 0.4U/kg used in the current study in comparison to 0.3U/kg in original study with healthy subjects. But the magnitude of difference can not only be attributed to the different doses used.

The differences between IAps and BIAps 70 are 26% and 38% for the PD endpoints, AUC_{GIR}, at early phase (0-2 hours) and intermediary phase (4-12 hours), respectively, which makes BIAsp 70 sufficiently distinct from IAsp.

The differences between BIAps 70 and BIAps 50 in PD parameters are minimal. 14% difference for the primary PD endpoint $AUC_{GIR\ 0\text{-}2\ hrs}$ between these two mixes was observed. But the statistical analysis indicated that the difference was not statistically significant. The $AUC_{GIR\ 4\text{-}12\ hrs}$ between BIAsp 70 and BIAsp 50 also showed the ratio of 1.04, which made these two insulin mixes almost superimposable in the intermediary phase.

The differences between BIAps 70 and BIAps 30 are 74% and 15% for the early phase (0-2 hours) and intermediary phase (4-12 hours), respectively as shown in PD endpoints. The greater difference in the early phase may be partially attributed by reduced bioavailability in BIAsp 30 (30% reduced bioavailability for BIAsp 30 than IAsp). The difference between BIAps 50 and BIAps 30 in PD parameters is similar to what was observed between BIAsp 70 and BIAsp 30. The difference in the ratio for the primary PD endpoint $AUC_{GIR\ 0\text{-}2\ hrs}$ between BIAsp 50 and BIAsp 30 was 52%. In the intermediary phase $AUC_{GIR\ 4\text{-}12\ hrs}$, the difference in the ratio is 18%. The difference between BIAsp 70 and BIAsp 30 should have been greater than that between BIAsp 50 and BIAsp 30 based on the composition of short- and long-acting insulins. However, the result indicated the opposite (15% vs 18%), suggesting great variations in response to these premixes in type 1 diabetic patients in this study.

Furthermore, PK analysis showed that the intermediary parameters $AUC_{IAsp\ 4\text{-}12\ hours}$ is indistinguishable for all three NovoLog mixes. The differences in $AUC_{IAsp\ 4\text{-}12\ hours}$ are 6% between BIAsp 70 and BIAsp 50, 9% between BIAsp 50 and BIAsp 30, and 5% between BIAsp 70 and BIAsp 30 and these are even smaller differences than those in the previous study conducted in healthy subjects (Study 1086).

These PK and PD results reveal that there are great variations within and between studies. It can be concluded that BIAsp 70 and BIAsp 50 are not distinct products from each other based on intermediary phase analysis. Whether the differences observed between BIAsp 70 or BIAsp 50 versus IAsp and BIAsp 30 may translate into clinical

benefits will depend on the evaluation of those supportive clinical trials, including phase 3 Study BIAsp-1440 being reviewed by the medical review team.

With regard to the DSI inspection, this reviewer did check on data for these seven subjects on four visits. This reviewer judged that these findings would not affect overall results as described in this review. A re-analysis may not generate additional values for the natures of the study. Also, NEFA profiles are used by the sponsor as secondary PD endpoints and the Agency does not use them as approval criteria. Therefore, the deficiency in NEFA assay will not impact the Agency's conclusion.

1. Trial 1746 Synopsis

2 Synopsis

Trial Registration ID-number Not applicable	**EudraCT number – EU only** 2005-004965-40
Title of Trial A Double-Blind, Randomised, Four-Period Crossover Trial Comparing the Pharmacodynamics and Pharmacokinetics after Single Dose of Biphasic Insulin Aspart 30, Biphasic Insulin Aspart 50, Biphasic Insulin Aspart 70 and Insulin Aspart in Subjects with Type 1 Diabetes	
Investigator Tim Heise, MD Profil Institute for Metabolic Research, Ltd, Neuss, Germany	
Trial Site Profil Institute for Metabolic Research, Ltd, Neuss, Germany	
Publications None	
Trial Period 19 April 2006 to 20 July 2006	**Development Phase** Phase 1
Objectives **Primary Objective:** • To compare the early pharmacodynamics of 0.4 U/kg BIAsp 30, 0.4 U/kg BIAsp 50, 0.4 U/kg BIAsp 70 and 0.4 U/kg IAsp, based on GIR results. **Secondary Objectives:** • To compare the pharmacokinetics of 0.4 U/kg BIAsp 30, 0.4 U/kg BIAsp 50, 0.4 U/kg BIAsp 70 and 0.4 U/kg IAsp in subjects with type 1 diabetes. • To compare other single dose pharmacodynamic properties as defined as standard GIR endpoints from the GIR curves between BIAsp 30, BIAsp 50, BIAsp 70 and IAsp. • To compare end of action as derived from the blood glucose concentrations during the clamp (end of action is defined as the time-point when blood glucose exceeds 160 mg/dL with no glucose infusion in the last 30 min or longer). • To compare pharmacodynamic properties derived from the serum NEFA concentrations between BIAsp 30, BIAsp 50, BIAsp 70 and IAsp. • To evaluate the safety of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp.	
Methodology • This was a randomised, single centre, four-period cross-over trial to compare the pharmacodynamics and pharmacokinetics after single dose administration of four different products (biphasic insulin aspart 30 (BIAsp 30), biphasic insulin aspart 50 (BIAsp 50), biphasic insulin aspart 70 (BIAsp 70) and insulin aspart (IAsp)) on four different occasions. • After successful screening, subjects were randomised to one of eight treatment sequences, selected from the 24 possible treatment sequences, involving subcutaneous (s.c.) single-dose administration of 0.4 U/kg BIAsp 30, 0.4 U/kg BIAsp 50, 0.4 U/kg BIAsp 70 and 0.4 U/kg IAsp, respectively, at four different dosing visits. • There were wash-out periods of 7-14 days between the four dosing visits. • At each dosing visit subjects received a controlled intravenous infusion of glucose and human soluble insulin (Actrapid®) for 4-6 hours prior to trial drug administration in order to keep the blood glucose concentration stable at a level of 90 mg/dL (5.0 mmol/L), i.e. a glucose clamp with a target blood glucose level of 90 mg/dL (5.0 mmol/L) was initiated. The glucose clamp was terminated after 12 hours post-dosing (IAsp) or 28 hours post-dosing (BIAsp 30, BIAsp 50 and BIAsp 70) or earlier if blood glucose levels increased to concentrations above 160 mg/dL (8.9 mmol/L) with no glucose infusion during the last 30 min.	

Methodology (continued)

- Blood samples for measurement of serum IAsp, serum nonesterified fatty acids and blood glucose were drawn before dosing and frequently after dosing for the entire duration of the glucose clamp.
- Safety assessments included adverse events, physical examination, vital signs, electrocardiograms (ECGs), clinical laboratory tests (haematology, biochemistry, urinalysis (incl. pregnancy test in females) and coagulation parameters), hypoglycaemic episodes and local tolerability at the injection site.

Number of Subjects Planned and Analysed

It was planned to randomise 32 subjects in order for 28 to complete the trial. All 32 subjects completed the trial and all 32 subjects were included in the pharmacokinetic, pharmacodynamic and safety analysis.

Diagnosis and Main Criteria for Inclusion

Men and women (non-smokers) with type 1 diabetes aged 18-55 years, with diabetes ≥ 12 months, BMI ≤ 32 kg/m^2, serum C-peptide ≤ 0.4 ng/mL, HbA_{1c} ≤ 9% and receiving basal-bolus treatment with soluble human insulin, insulin lispro, insulin glulisine, neutral protamine hagedorn insulin, insulin detemir or insulin glargine.

Test Product, Dose and Mode of Administration, Batch Number

Single dose (0.4 U/kg) of BIAsp 30 (Batch No. RQ50579), BIAsp 50 (Batch No. PQ50709), BIAsp 70 (Batch No. PQ50675) or IAsp (Batch No. RQ50660), 100 U/mL, delivered in Penfill® 3.0 mL cartridges and injected s.c. in the umbilical region by using Micro-Fine™ syringes.

Duration of Treatment

Four single doses of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp, respectively, administered at four different occasions at intervals of 7-14 days.

Reference Therapy, Dose and Mode of Administration, Batch Number

There was no reference therapy. The four test products were compared.

Criteria for Evaluation – Efficacy

Pharmacokinetics:

- Serum IAsp concentrations for 28 hours (BIAsp 30, BIAsp 50 and BIAsp 70) or 12 hours (IAsp) following a single dose of either BIAsp 30, BIAsp 50, BIAsp 70 or IAsp.

Pharmacodynamics:

- Glucose infusion rate (GIR) during a euglycaemic clamp for 28 hours (BIAsp 30, BIAsp 50 and BIAsp 70) or 12 hours (IAsp) following a single dose of either BIAsp 30, BIAsp 50, BIAsp 70 or IAsp.
- Blood glucose and serum NEFA concentrations before dosing and for the entire duration of the glucose clamp.

Criteria for Evaluation – Safety

The safety evaluation was based on adverse events, physical examination, vital signs, ECG, clinical laboratory tests (haematology, biochemistry, urinalysis and coagulation parameters), hypoglycaemic episodes and local tolerability at the injection site.

Statistical Methods

Primary Endpoint:

- $AUC_{GIR,0\text{-}2h}$, the area under the curve (AUC) of the GIR curve from 0 to 2 hours

Pairwise tests for no difference between treatments in $AUC_{GIR,0\text{-}2h}$ were carried out using an analysis of variance (ANOVA) model with a fixed treatment effect, a fixed period effect, a random subject effect and a measurement error. $AUC_{GIR,0\text{-}2h}$ was log-transformed before analysis and the estimated treatment differences were back transformed to the original scale to yield estimates of treatment ratios. A p-value of 5% was used as the level of significance.

Secondary Endpoints:

Serum Insulin Aspart

- C_{max}, the maximal IAsp concentration during the glucose clamp
- $t_{max,IAsp}$, the time of the maximal IAsp concentration during the glucose clamp

Statistical Methods (continued)

- $AUC_{IAsp,0-2h}$, the AUC of the IAsp curve from 0 to 2 hours
- $AUC_{IAsp,0-4h}$, the AUC of the IAsp curve from 0 to 4 hours
- $AUC_{IAsp,0-6h}$, the AUC of the IAsp curve from 0 to 6 hours
- $AUC_{IAsp,0-12h}$, the AUC of the IAsp curve from 0 to 12 hours
- $AUC_{IAsp,0-28h}$, the AUC of the IAsp curve from 0 to 28 hours
- $AUC_{IAsp,0-inf}$, the AUC of the IAsp curve from 0 to infinity
- $AUC_{IAsp,4-12h}$, the AUC of the IAsp curve from 4 to 12 hours
- $AUC_{IAsp,6-12h}$, the AUC of the IAsp curve from 6 to 12 hours
- $AUC_{IAsp,6-28h}$, the AUC of the IAsp curve from 6 to 28 hours
- $AUC_{IAsp,12-28h}$, the AUC of the IAsp curve from 12 to 28 hours
- $t_{½}$, the terminal half-life of the IAsp concentration

Glucose Infusion Rate

- GIR_{max}, the maximal GIR during the glucose clamp
- $t_{max,GIR}$, the time of the maximal GIR during the glucose clamp
- $AUC_{GIR,0-4h}$, the AUC of the GIR curve from 0 to 4 hours
- $AUC_{GIR,0-6h}$, the AUC of the GIR curve from 0 to 6 hours
- $AUC_{GIR,0-12h}$, the AUC of the GIR curve from 0 to 12 hours
- $AUC_{GIR,0-28h}$, the AUC of the GIR curve from 0 to 28 hours
- $AUC_{GIR,4-12h}$, the AUC of the GIR curve from 4 to 12 hours
- $AUC_{GIR,6-12h}$, the AUC of the GIR curve from 6 to 12 hours
- $AUC_{GIR,6-28h}$, the AUC of the GIR curve from 6 to 28 hours
- $AUC_{GIR,12-28h}$, the AUC of the GIR curve from 12 to 28 hours
- $t_{GIR,10\%\,AUC}$, the time t when $AUC_{GIR,0-t}$ equals 10% of $AUC_{GIR,0-28h}$
- $t_{GIR,90\%\,AUC}$, the time t when $AUC_{GIR,0-t}$ equals 90% of $AUC_{GIR,0-28h}$

Blood Glucose Escape

- $tGIR_0$, the time until GIR declined to zero defined as the last time-point of the last 5 min interval where GIR > 0.02 mg/kg/min
- BG $escape_{90}$, the time until blood glucose (BG) started to escape from the clamp target level of 90 mg/dL (5.0 mmol/L)
- BG $escape_{140}$, the time until blood glucose exceeded 140 mg/dL (7.8 mmol/L) with no glucose infusion during the last 30 min
- BG $escape_{160}$, the time until blood glucose exceeded 160 mg/dL (8.9 mmol/L) with no glucose infusion during the last 30 min

Serum Nonesterified Fatty Acids

- $C_{min,NEFA}$, the minimum NEFA concentration during the glucose clamp
- $t_{min,NEFA}$, the first time of minimum NEFA concentration during the glucose clamp
- $AUC_{NEFA,0-2h}$, the AUC of the NEFA curve from 0 to 2 hours
- $AUC_{NEFA,0-4h}$, the AUC of the NEFA curve from 0 to 4 hours
- $AUC_{NEFA,0-6h}$, the AUC of the NEFA curve from 0 to 6 hours
- $AUC_{NEFA,0-12h}$, the AUC of the NEFA curve from 0 to 12 hours
- $AUC_{NEFA,0-28h}$, the AUC of the NEFA curve from 0 to 28 hours
- $AUC_{NEFA,4-12h}$, the AUC of the NEFA curve from 4 to 12 hours
- $AUC_{NEFA,6-12h}$, the AUC of the NEFA curve from 6 to 12 hours
- $AUC_{NEFA,6-28h}$, the AUC of the NEFA curve from 6 to 28 hours
- $AUC_{NEFA,12-28h}$, the AUC of the NEFA curve from 12 to 28 hours

Statistical Methods (continued)

The endpoints $AUC_{IAsp,0-2h}$, $AUC_{IAsp,0-4h}$ $AUC_{IAsp,0-6h}$, $AUC_{IAsp,0-12h}$, $AUC_{IAsp,0-28h}$, $AUC_{IAsp,0-inf}$, $AUC_{IAsp,4-12h}$, $AUC_{IAsp,6-12h}$, $AUC_{IAsp,6-28h}$, $AUC_{IAsp,12-28h}$, C_{max}, $AUC_{GIR,0-4h}$ $AUC_{GIR,0-6h}$, $AUC_{GIR,0-12h}$, $AUC_{GIR,0-28h}$, $AUC_{GIR,4-12h}$, $AUC_{GIR,6-12h}$, $AUC_{GIR,6-28h}$, $AUC_{GIR,12-28h}$, GIR_{max}, $AUC_{NEFA,0-2h}$, $AUC_{NEFA,0-4h}$, $AUC_{NEFA,0-6h}$, $AUC_{NEFA,0-12h}$, $AUC_{NEFA,0-28h}$, $AUC_{NEFA,4-12h}$, $AUC_{NEFA,6-12h}$, $AUC_{NEFA,6-28h}$, $AUC_{NEFA,12-28h}$ and $C_{min,NEFA}$ were log-transformed and analysed using an ANOVA model as for the primary endpoint. The endpoints $tGIR_0$, BG $escape_{90}$, BG $escape_{140}$ and BG $escape_{160}$ were analysed by a survival approach. An Accelerated Failure Time (AFT) model was estimated based on a Weibull distribution. The AFT approach was chosen to enable a comparison between treatments as a ratio of these four endpoints. The endpoints $t_{max,GIR}$, $t_{GIR,10\%\,AUC}$, $t_{GIR,90\%\,AUC}$, $t_{max,IAsp}$, $t_{1/2}$ and $t_{min,NEFA}$ were summarised but not formally analysed.

Demography of Trial Population

The 32 subjects with type 1 diabetes (11 females and 21 males) were 31 Caucasians and 1 black or African American. Their mean age was 37 years (range 21 to 52 years), mean weight was 76 kg (range 58 to 96 kg), mean BMI was 25 kg/m^2 (range 20 to 31 kg/m^2), mean HbA_{1c} was 7.6% (range 6.3 to 8.5%), C-peptide was ≤ 0.14 nmol/L in all subjects and they had a mean diabetes duration of 19 years (range 5 to 33 years).

Efficacy Results

- The primary endpoint, $AUC_{GIR,0-2h}$, increased with increasing fraction of soluble IAsp. $AUC_{GIR,0-2h}$ was 26% higher with IAsp than with BIAsp 70 and 52% higher with BIAsp 50 than with BIAsp 30, while the 14% higher $AUC_{GIR,0-2h}$ with BIAsp 70 than with BIAsp 50 did not reach statistical significance.
- The maximum serum IAsp concentration, C_{max}, and the maximum glucose infusion rate, GIR_{max}, both increased significantly with increasing fraction of soluble IAsp.
- The two endpoints, $t_{max,IAsp}$ and $t_{max,GIR}$ were both similar between the four IAsp preparations, i.e. the time of maximum serum IAsp concentration and the time of maximum metabolic activity did not depend on the fraction of soluble IAsp.
- Early IAsp exposure increased significantly with increasing fraction of soluble IAsp. The three endpoints, $AUC_{IAsp,0-2h}$, $AUC_{IAsp,0-4h}$ and $AUC_{IAsp,0-6h}$, all differed significantly between IAsp and BIAsp 70, between BIAsp 70 and BIAsp 50 and between BIAsp 50 and BIAsp 30.
- Early metabolic activity of IAsp, in terms of glucose lowering effect, increased significantly with increasing fraction of soluble IAsp. The two endpoints, $AUC_{GIR,0-4h}$ and $AUC_{GIR,0-6h}$, both differed significantly between IAsp and BIAsp 70, between BIAsp 70 and BIAsp 50 and between BIAsp 50 and BIAsp 30 (see above for findings on the primary endpoint, $AUC_{GIR,0-2h}$).
- Late IAsp exposure was higher with the three BIAsp preparations than with IAsp. Between 6 and 12 hours after trial drug administration ($AUC_{IAsp,6-12h}$), serum IAsp concentration was 63% lower with IAsp compared with BIAsp 70. Between 12 and 28 hours after trial drug administration ($AUC_{IAsp,12-28h}$), serum IAsp concentration was 32% lower with BIAsp 50 compared with BIAsp 30. There was no significant difference in $AUC_{IAsp,12-28h}$ between BIAsp 70 and BIAsp 50.
- Late metabolic activity of IAsp, in terms of glucose lowering effect (AUC_{GIR}) and lipolytic inhibitory effect (AUC_{NEFA}), increased with increasing fraction of protaminated IAsp. $AUC_{GIR,6-12h}$ was 86% lower with IAsp compared with BIAsp 70. $AUC_{NEFA,6-12h}$ was 66% higher with IAsp compared with BIAsp 70, 29% higher with BIAsp 70 compared with BIAsp 50 and 40% higher with BIAsp 50 compared with BIAsp 30. Moreover, $AUC_{GIR,12-28h}$ and $AUC_{NEFA,12-28h}$ both differed significantly between the three BIAsp preparations.
- End of metabolic action, as evidenced by the four endpoints $tGIR_0$, BG $escape_{90}$, BG $escape_{140}$ and BG $escape_{160}$, occurred significantly later with increasing fraction of protaminated IAsp.

Safety Results

- There were a total of 20 AEs occurring in 34% of the trial population (11 subjects).
- Fourteen (14) AEs were mild, 5 were moderate and 1 was severe. The latter was a circulatory collapse occurring 4 hours after administration of BIAsp 30. This AE lasted 10 min, was assessed to be possibly related to trial product and the subject recovered and completed the trial.
- Sixteen (16) AEs were assessed to be unlikely related to trial product, while 4 AEs were assessed to be possibly related to trial product.

Safety Results (continued) • The most frequent AE was headache (12 AEs). Other AEs were gastrointestinal disorders (5), nasopharyngitis (1), hyperglycaemia (1) and circulatory collapse (1). • There was a higher incidence of AEs following administration of BIAsp 30 (60% of all AEs) compared with BIAsp 50 (10%), BIAsp 70 (10%) and IAsp (20%). • There were no clinically relevant findings for biochemistry, haematology, urinalysis, coagulation parameters, vital signs, ECG, physical examination, hypoglycaemic episodes and local tolerability at the injection site. • In general, single-dose administration of BIAsp 30, BIAsp 50, BIAsp 70 and IAsp was well tolerated in subjects with type 1 diabetes.
Conclusions • Glucose lowering action in the early phase increased significantly with increasing fraction of soluble IAsp. • Early phase pharmacokinetic properties were significantly different between BIAsp 30, BIAsp 50, BIAsp 70 and IAsp. • Serum IAsp concentrations and glucose lowering action between 6 and 12 hours after trial drug administration were lower with IAsp compared with the three BIAsp preparations. • The inhibitory effect of IAsp on lipolysis differed significantly between all four trial products between 6 and 12 hours after trial drug administration as evidenced by serum NEFA levels. • Between 12 and 28 hours after trial drug administration, the glucose lowering effect and the inhibition of lipolysis induced by IAsp were both significantly higher with increasing fraction of protaminated IAsp. Within this time frame, a higher serum IAsp concentration was seen with BIAsp 30 compared with BIAsp 50. • End of metabolic action occurred significantly later with increasing fraction of protaminated IAsp. • No safety concerns were raised.
The trial was conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice.

Glucose Clamp:

Subjects started receiving an i.v. infusion of glucose and human soluble insulin (Actrapid®) at the latest 4-6 hours prior to trial drug administration in order to keep the blood glucose concentration stable at a level of 90 mg/dL (5.0 mmol/L). The glucose and insulin infusion was controlled by a Biostator. The subjects remained fasting but were allowed to drink water *ad libitum*, and stayed in a supine or semi-supine position during the entire glucose clamp. For the Biostator's blood glucose concentration measurements, a catheter was inserted into a wrist or hand vein of the left arm and connected to the glucose sensor of the Biostator. The left hand remained in a 'Hot-Box' (55°C) throughout the trial, resulting in an arterialisation of the venous blood. Another catheter was inserted into a vein in the right forearm for infusion of a 20% glucose solution as well as a human soluble insulin solution during the baseline period. Bolus injections of i.v. human insulin could also be performed in order to reach the blood glucose target level, but no bolus injection of insulin was allowed during the last 3 hours before trial drug administration. From 3 hours prior to trial drug administration and onwards, the insulin infusion rate was not allowed to exceed 0.2 mU/kg/min. From one hour prior to trial drug administration, the insulin infusion rate was decreased as much as possible, so that the blood glucose target level of 90 mg/dL was kept with as little glucose infusion as possible. The i.v. insulin infusion was terminated completely 30 min before trial drug administration. Following trial drug administration, blood glucose concentration was kept constant at the target level (90 mg/dL) by means of an adjustable i.v. infusion of glucose through the Biostator, which automatically calculated the appropriate adjustments of the GIR. The blood glucose measurements of the Biostator were checked against blood glucose measurements performed by an autoanalyser (see below). The glucose clamp lasted for 12 hours after IAsp administration and for 28 hours after administration of BIAsp 30,

BIAsp 50 and BIAsp 70. The glucose clamp was terminated early if blood glucose levels increased to concentrations above 160 mg/dL (8.9 mmol/L), with no glucose infusion during the last 30 minutes, to secure that the full metabolic effect of the trial drug had been captured.

Table 9–4 Dosing Visit Flow Chart

Approximate hour[1]	Nominal timing	Insulin/glucose i.v. infusion	Blood sampling for IAsp and NEFA	Glucose check during clamp
Minimum 08:00-12:00 Maximum 08:00-14:00	At between -360 min and -240 min	Start of i.v. glucose and i.v. Actrapid® infusion Blood glucose target at 90 mg/dL		At least every 30 min
09:00	-180 min	Last bolus i.v. of Actrapid®		
11:00	-60 min	X[4]		
11:45	-30 min	Stop i.v. Actrapid® infusion	X	
12:00	0 min	Subcutaneous administration of trial drug	X[2]	
	15 min		X	
	30 min		X	
	45 min		X	
13:00	1h		X	
	1h 20min		X	
	1h 40 min		X	
14:00	2h		X	
14:30	2h 30min		X	
15:00	3h		X	
15:30	3h 30min		X	
16:00	4h		X	
18:00	6h		X	
20:00	8h		X	
24:00	12h	Stop clamp procedure with IAsp[3]	X	
04:00	16h		X	
08:00	20h		X	
12:00	24h		X	
16:00	28h	Stop clamp procedure with BIAsp[3]	X	

1. Actual time could vary but it was ensured that trial drug administration occurred no later than 15:00 hours.
2. Blood sample was drawn within 5 minutes before injection of the trial product.
3. The glucose clamp procedure was terminated earlier than 12 (IAsp) or 28 (BIAsp) hours post-dose, if blood glucose was >160 mg/dL (8.9 mmol/L) with no glucose infusion during the last 30 min.
4. Average glucose infusion rate during the last 60 min pre-dose should not exceed 1 mg/kg/min.

14.2.3: Analysis of IAsp endpoints, PK Analysis Set

stat	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIAsp70	BIAsp70 / BIAsp50	BIAsp50 / BIAsp30	BIAsp70 / BIAsp30
AUC IAsp 0-2h(mU*h/L)								
N	31	31	31	31				
Mean	261	192	130	87				
Ratio					1.36	1.48	1.48	2.20
CI					(1.23, 1.50)	(1.34, 1.63)	(1.35, 1.64)	(1.99, 2.42)
Prob>T					<.0001	<.0001	<.0001	<.0001
AUC IAsp 0-4h(mU*h/L)								
N	31	31	31	31				
Mean	460	337	239	172				
Ratio					1.36	1.41	1.39	1.96
CI					(1.27, 1.46)	(1.32, 1.52)	(1.29, 1.49)	(1.83, 2.11)
Prob>T					<.0001	<.0001	<.0001	<.0001
AUC IAsp 0-6h(mU*h/L)								
N	31	31	31	31				
Mean	530	407	294	224				
Ratio					1.30	1.38	1.32	1.82
CI					(1.22, 1.39)	(1.29, 1.48)	(1.23, 1.41)	(1.70, 1.94)
Prob>T					<.0001	<.0001	<.0001	<.0001
AUC IAsp 0-12h(mU*h/L)								
N	31	31	31	31				
Mean	570	490	383	326				
Ratio					1.16	1.28	1.18	1.51
CI					(1.08, 1.25)	(1.19, 1.37)	(1.10, 1.26)	(1.40, 1.62)
Prob>T					<.0001	<.0001	<.0001	<.0001
AUC IAsp 0-28h(mU*h/L)								
N	31	31	31	31				
Mean	575	536	439	401				
Ratio					1.07	1.23	1.09	1.34
CI					(1.00, 1.15)	(1.14, 1.32)	(1.01, 1.18)	(1.24, 1.44)
Prob>T					0.0647	<.0001	0.0229	<.0001

Comparison by linear model with treatment and visit as fixed effects and subject as random effect.
Endpoint is log-transformed before analysis and back-transformed to yield ratio estimates.
Means are LS Means estimated from the model. Prob>T: Significance probability

Analysis of IAsp endpoints, PK Analysis Set

stat	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIAsp70	BIAsp70 / BIAsp50	BIAsp50 / BIAsp30	BIAsp70 / BIAsp30
AUC IAsp 0-inf(mU*h/L)								
N	31	31	31	31				
Mean	589	564	474	442				
Ratio					1.04	1.19	1.07	1.28
CI					(0.96, 1.14)	(1.09, 1.29)	(0.99, 1.17)	(1.17, 1.39)
Prob>T					0.3080	<.0001	0.1012	<.0001
AUC IAsp 4-12h(mU*h/L)					—			
N	31	31	31	31				
Mean	90	142	136	150				
Ratio					0.63	1.04	0.91	0.95
CI					(0.54, 0.74)	(0.89, 1.22)	(0.78, 1.07)	(0.81, 1.11)
Prob>T					<.0001	0.6060	0.2451	0.5229
AUC IAsp 6-12h(mU*h/L)								
N	31	31	31	31				
Mean	29	77	82	99				
Ratio					0.37	0.94	0.84	0.78
CI					(0.30, 0.46)	(0.76, 1.16)	(0.68, 1.03)	(0.63, 0.97)
Prob>T					<.0001	0.5379	0.0944	0.0233
AUC IAsp 6-28h(mU*h/L)								
N	31	31	31	31				
Mean	32	116	130	170				
Ratio					0.28	0.90	0.75	0.68
CI					(0.22, 0.34)	(0.73, 1.11)	(0.62, 0.91)	(0.55, 0.85)
Prob>T					<.0001	0.3082	0.0129	0.0006
AUC IAsp 12-28h(mU*h/L)								
N		31	31	31				
Mean		36	49	63				
Ratio						0.83	0.68	0.57
CI						(0.65, 1.05)	(0.53, 0.86)	(0.45, 0.72)
Prob>T						0.1143	0.0020	<.0001

Comparison by linear model with treatment and visit as fixed effects and subject as random effect.
Endpoint is log-transformed before analysis and back-transformed to yield ratio estimates.
Means are LS Means estimated from the model. Prob>T: Significance probability

14.2.8: Analysis of AUC GIR 0-2h

	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIAsp70	BIAsp70 / BIAsp50	BIAsp50 / BIAsp30	BIAsp70 / BIAsp30
PD Analysis Set								
AUC GIR 0-2h(mg/kg/min)								
CI					(1.08, 1.47)	(0.98, 1.33)	(1.31, 1.78)	(1.49, 2.03)
Mean	540	429	376	247				
N	31	31	31	31				
Prob>T					0.0038	0.0933	<.0001	<.0001
Ratio					1.26	1.14	1.52	1.74
All Profiles Included								
AUC GIR 0-2h(mg/kg/min)								
CI					(1.08, 1.46)	(1.00, 1.35)	(1.30, 1.75)	(1.50, 2.03)
Mean	540	429	370	246				
N	32	32	32	32				
Prob>T					0.0029	0.0523	<.0001	<.0001
Ratio					1.26	1.16	1.51	1.75

14.2.9: Analysis of Secondary GIR endpoints, PD Analysis Set

stat	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIAsp70	BIAsp70 / BIAsp50	BIAsp50 / BIAsp30	BIAsp70 / BIAsp30
AUC GIR 0-4h(mg/kg)								
N	31	31	31	31				
Mean	1416	1174	1001	725				
Ratio					1.21	1.17	1.38	1.62
CI					(1.09, 1.35)	(1.05, 1.32)	(1.23, 1.55)	(1.44, 1.82)
Prob>T					0.0017	0.0072	<.0001	<.0001
AUC GIR 0-6h(mg/kg)								
N	31	31	31	31				
Mean	1901	1652	1396	1094				
Ratio					1.15	1.18	1.28	1.51
CI					(1.05, 1.27)	(1.08, 1.30)	(1.16, 1.40)	(1.37, 1.66)
Prob>T					0.0041	0.0006	<.0001	<.0001
AUC GIR 0-12h(mg/kg)								
N	31	31	31	31				
Mean	2061	2124	1909	1777				
Ratio					0.97	1.11	1.07	1.19
CI					(0.87, 1.08)	(1.00, 1.24)	(0.97, 1.19)	(1.07, 1.33)
Prob>T					0.5778	0.0482	0.1928	0.0012
AUC GIR 0-28h(mg/kg)								
N	31	31	31	31				
Mean	2060	2141	1972	1914				
Ratio					0.96	1.09	1.03	1.12
CI					(0.86, 1.07)	(0.97, 1.21)	(0.92, 1.15)	(1.00, 1.25)
Prob>T					0.4930	0.1440	0.5869	0.0466
AUC GIR 6-12h(mg/kg)								
N	31	31	31	31				
Mean	543	876	846	1029				
Ratio					0.62	1.04	0.82	0.85
CI					(0.50, 0.77)	(0.84, 1.28)	(0.67, 1.01)	(0.69, 1.05)
Prob>T					<.0001	0.7412	0.0665	0.1307
AUC GIR 6-12h(mg/kg)								
N	29	31	30	31				
Mean	51	356	497	661				

Comparison by linear model with treatment and visit as fixed effects and subject as random effect.
Endpoint is log-transformed before analysis and back-transformed to yield ratio estimates.
Means are LS Means estimated from the model. Prob>T: Significance probability
xl4-1746/freeze_20061212 - 12DEC2006 - t_ana_gir_sec.sas/t_ana_gir_sec.txt

Analysis of Secondary GIR endpoints, PD Analysis Set

stat	IAsp	BIAsp 70	BIAsp 50	BIAsp 30	IAsp / BIAsp70	BIAsp70 / BIAsp50	BIAsp50 / BIAsp30	BIAsp70 / BIAsp30
Ratio					0.14	0.72	0.75	0.54
CI					(0.07, 0.29)	(0.36, 1.44)	(0.37, 1.51)	(0.27, 1.08)
Prob>T					<.0001	0.3451	0.4207	0.0799
AUC GIR 6-28h(mg/kg)								
N	29	31	30	31				
Mean	51	367	544	786				
Ratio					0.14	0.67	0.69	0.47
CI					(0.07, 0.29)	(0.33, 1.36)	(0.34, 1.40)	(0.23, 0.94)
Prob>T					<.0001	0.2676	0.3006	0.0325
AUC GIR 12-28h(mg/kg)								
N		29	28	31				
Mean		1	7	40				
Ratio						0.21	0.17	0.04
CI						(0.06, 0.72)	(0.05, 0.57)	(0.01, 0.12)
Prob>T						0.0139	0.0050	<.0001
GIR Max (mg/kg/min)								
N	31	31	31	31				
Mean	9.6	6.8	5.8	4.5				
Ratio					1.27	1.18	1.29	1.52
CI					(1.15, 1.40)	(1.06, 1.30)	(1.17, 1.43)	(1.37, 1.68)
Prob>T					<.0001	0.0018	<.0001	<.0001

12 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Xiao-xiong Wei
6/15/2007 09:23:51 PM
BIOPHARMACEUTICS

Sally Choe
6/18/2007 07:44:27 AM
BIOPHARMACEUTICS

62 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)