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APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-172

Pharmacology Review(s)

NDA 21-172

Addendum to Pharmacologist's labeling review of NDA 21-172

LABELING

The following pharm/tox labeling changes are recommended in the sponsor's 2/9/01 submission:

Carcinogenicity, Mutagenicity, Impairment of Fertility
Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of NovoLog Mix 70/30. In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with [redacted] NovoLog®, the rapid-acting component of NovoLog Mix 70/30, at 10, 50, and 200 U/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area, respectively). At a dose of 200 U/kg/day, [redacted] NovoLog® increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for [redacted] NovoLog® was not significantly different than for regular human insulin. The relevance of these findings to humans is not known. [redacted] NovoLog® was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, [redacted] NovoLog® at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area) had no direct adverse effects on male and female fertility, or on general reproductive performance of animals.

Pregnancy: Teratogenic Effects: Pregnancy Category C:
Animal reproduction studies have not been conducted with NovoLog Mix 70/30. It is not known whether NovoLog Mix 70/30 can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. [redacted]

[redacted]

The effects of [redacted] NovoLog® did not differ from those observed with subcutaneous regular human insulin. [redacted] NovoLog®, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32-times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area), and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/kg/day for rats and equal to the human subcutaneous dose of 1.0 U/kg/day for rabbits based on U/body surface area. [redacted]

Recommendation:
From the preclinical standpoint, approval of this application is recommended, pending acceptable labeling modifications.

Indra Antonipillai, Ph.D.
Pharmacologist, HFD-510

cc: NDA Arch
HFD510
HFD510/antonipillai/elhage/koller/jrhee
Review Code: AP
Filename:nda21172a2

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

/s/

Indra Antonipillai
10/15/01 10:46:02 AM
PHARMACOLOGIST
Approval of this application is recommended, pending labeling changes.
Please communicate the labeling changes to the sponsor
The labeling changes need to be communicated to the sponsor

Jeri El Hage
10/17/01 12:42:14 PM
PHARMACOLOGIST

Addendum to the Pharmacologist's August 24, 2000 review of NDA 21-172

LABELING

Following changes in labeling are made to clearly state that the studies under "**Carcinogenicity, Mutagenicity, Impairment of Fertility** and '**Pregnancy**' were carried out with Novolog, the rapid-acting component of ——— 70/30, which was not stated in the pharmacology review of August 24, 2000:

The label for ——— 70/30 should read as follows:

Carcinogenicity, Mutagenicity, Impairment of Fertility

———**Standard 2-year carcinogenicity** studies in animals have not been performed to evaluate the carcinogenic potential of 70/30. In 52 week studies, **Sprauge-Dawley** rats **were** ——— dosed subcutaneously with **NovoLog, the rapid-acting component of 70/30** ——— ——— at ——— **at 10, 50, and** 200 U/kg/day (approximately **2, 8, and** 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area, respectively). At a dose of 200 U/kg/day, NovoLog **increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors for NovLog was not significantly different than for regular human insulin. The relevance of these findings to humans is not known.** ——— ——— NovoLog was not genotoxic in the following ——— ——— tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, **NovoLog** at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), **had** no direct adverse effects on male and female fertility, or **on** general reproductive performance of animals ———

Pregnancy: Teratogenic effects: Pregnancy category **C**: ——— and teratology studies have been performed with **NovoLog (the rapid-acting component of ——— 70/30)** and regular human insulin in rats **and rabbits. In these studies, NovoLog was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis.**

. The effects of **NovoLog** is ——— did not ——— differ from

those observed with subcutaneous regular human insulin
NovoLog, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32-times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area), and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/kg/day for rats and equal to the human subcutaneous dose of 1.0 U/kg/day for rabbits, based on U/body surface area.

/S/

Indra Antonipillai, Ph.D.
Pharmacologist, HFD-510

/S/
9/22/00

cc: NDA Arch
HFD510
HFD510/antonipillai/elhage/koller/jrhee
Review Code: AP
Filename:21172a1

APPEARS THIS WAY
ON ORIGINAL

NDA 21-172

Review completed: August 24, 1999

Sponsor: Novo Nordisk Pharmaceuticals Inc., 100 Overlook Center, Princeton, NJ.

Date Submitted: December 17, 1999

Date Received: December 23, 1999.

Drug Class: Biphasic Insulin Aspart 30 ——— 70/30 injection), 30% soluble insulin aspart and 70% protamine crystallized insulin aspart, 100 U/ml SC injection.

Category: Insulin analog (Recombinant human insulin, DNA origin, β^{28} Asp-Insulin).

Indication: Treatment of diabetes (type I and 2).

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/S/

Indra Antonipillai, Ph.D.

S 8/24/00

cc: NDA Arch
HFD-510
HFD-510/elhage/antonipillai/koller/jrhee
File name: nda21172, Aspart 30, review #2

APPEARS THIS WAY
ON ORIGINAL

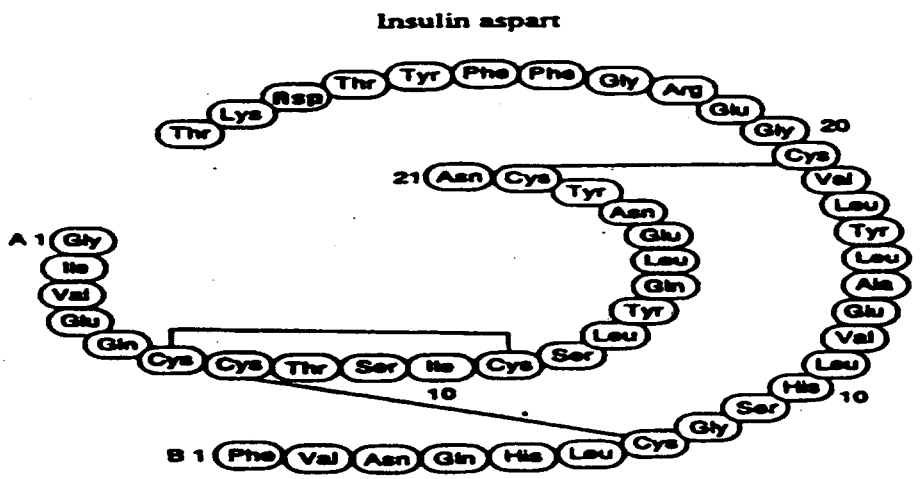
REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Insulin, diabetes, glucose
Reviewer Name: Indra Antonipillai
Division Name: Division of Metabolic and Endocrine Drug products.
HFD# 510
Review Completion Date: August 30, 2000

IND/NDA number: NDA 21-172
Serial number/date/type of submission: December 17, 1999, original application
Information to Sponsor: Yes (X) No ( ) - (labeling)
Sponsor or agent: Novo Nordisk Pharmaceuticals Inc., 100 Overlook Center, Princeton, NJ.
Manufacturer (if different) for drug substance: Novo Nordisk A/S, Novo Alle, DK-2880 Bagsvaerd, Denmark.

Drug:
Code Name: Biphasic Insulin Aspart 30[redacted] 70/30 injection, rDNA origin)
It contains 30% soluble insulin aspart and 70% protamine crystallized insulin aspart, it has a rapid onset and intermediate duration of action.

Generic Name: Biphasic Insulin Aspart.
Trade Name: N/A
Chemical Name: Biphasic Insulin Aspart 30 (Recombinant human insulin, DNA origin, β^{28} Asp-Insulin))
CAS Registry Number (if provided by sponsor): N/A
Molecular Formula/ Molecular Weight: $C_{256}H_{381}N_{65}O_{79}S_6$/5825.8
Structure:



Relevant INDs/NDAs/DMFs:
IND [redacted] (insulin X14, Novo Nordisk Pharmaceutical Inc)
NDA 19-938 (S/021), and NDA 20-986 (insulin apart, Novolog)

Drug Class: Insulin Analog
Indication: Treatment of diabetes (type I and 2) for control of hyperglycemia
Clinical formulation (and components): [redacted], 70/30 injection, 100 Units/ml of the active drug (insulin aspart) contains the following, at pH of 7.2-7.44:

Mannitol	36.4 mg/ml
Phenol	1.5 mg/ml
Metacresol	1.72 mg/ml
zinc	32.7 µg/ml
Disodium hydrogen phosphate dihydrate	1.25 mg/ml
Sodium chloride	0.58 mg/ml
Protamine sulfate	0.33 mg/ml
Sodium hydroxide	
Hydrochloric acid	

It is supplied as 10 ml vial, PenFill -3 ml cartridges, and Prefilled-3 ml syringes. Novolet is the approved name for prefilled syringe in Europe. The [redacted] 70/30 PenFill cartridges are for use with NovoPen 3 insulin delivery devices and novofine needles.

Route of administration: subcutaneous injection.

Proposed clinical protocol or use: [redacted] 70/30 (Insulin aspart) is indicated for the treatment of diabetes mellitus as a rapid acting insulin analog. It has a rapid onset and shorter duration of action than regular human insulin. [redacted] 70/30 is more rapid in its onset than biphasic human insulin 70/30 due to its faster absorption. The overall aim is to develop an insulin that provides metabolic control as good as biphasic human insulin 70/30, with an improved post-prandial glucose control. This product is intended for people with diabetes who use a pre-mixed insulin. The individual insulin aspart requirement is usually between 0.5-1 U/kg/day, but in more insulin resistant individuals, the requirement may increase up to 2 U/kg/day.

Introduction and drug history: The active ingredient in [redacted] 70/30 is insulin aspart (X-14 or NovoLog), which was approved in June 2000.

Studies reviewed within this submission:

Studies not reviewed within this submission: None.

PHARMACOLOGY

A. INTRODUCTION

[redacted]70/30 is a new formulation of insulin aspart. Insulin Aspart (X14, or Novolog) was approved for marketing in June 2000. It is an analog of human insulin in which the amino acid, proline, in position β28, has been replaced by aspartic acid. This modification was designed to decrease the self-association of the molecule. Upon subcutaneous injection, the hexamer of insulin aspart dissociates into monomer form more rapidly than human insulin, which leads to the fast onset of action, without losing other properties of human insulin.

Insulin aspart is produced by recombinant DNA technology in the yeast sacharomyces cerevisiae by Novo Nordisk A/S. The host strain is identical to the host strain used for the production of human insulin.

The present submission [redacted]70/30) provides for new formulation of this product for more rapid onset, than biphasic human insulin 70/30 (HI30), due to its faster absorption.[redacted]70/30 is a suspension, which contains 30% soluble insulin aspart and 70% insulin aspart protamine crystals. Biphasic insulin aspart 30 is being developed in order to present a premixed formulation with short acting and protracted insulin aspart. The primary difference of this product compared to the approved Novolog (insulin aspart) is that of the time-action profile. Given the extensive preclinical work done for the original NDA 20-986, limited preclinical studies have been provided in this NDA.

B. PHARMACOLOGY AND PHARMACOKINETICS

In Vivo studies

1. Glucose lowering effects of the biphasic insulin aspart 30 vs that of the biphasic human insulin 30 (HI30) in female crossbred LYYD pigs (Study # 960078):

Methods: Pigs have close similarities to humans with respect to composition of subcutaneous tissue, therefore this species was chosen for the current study. Eight fasted pigs (mean weight 97.6 kg, age 3 months old), in a single dose cross-over study received 20 U of insulin aspart 30 (batch # Q95014), or human insulin 30 (HI 30, batch # Q95013) in an ear, 5 mm into subcutis (by SC injection, using ^{125}I-Tyr A14 aspart or insulin) on each side of neck, on two subsequent study days, and plasma samples were obtained at 10-360 minutes. This formulation of insulin aspart 30 contained mannitol with —/Zn ——, while human insulin 30 (HI30) contained — Zn/ —— (HM PenMix 30). Also note that this formulation ——

The specific insulin aspart —— method was used to assay the drug levels.

Results: Insulin aspart 30 showed faster onset of action as it reached the maximum effect earlier and exerted more pronounced effect at the peak level. The largest differences between two insulins were in the mean values of time to minimum blood glucose, and the change in blood glucose conc. at 30 min. The time to minimum blood glucose (T_{min}) was shorter for Aspart 30 than for HI30 (129 vs 176 min, $p<0.05$). Blood glucose differences (delta) were higher for aspart 30 than for HI30 (-1.05 vs –0.2 mM, $p<0.05$, the mean base-line glucose values at 0 time for both Aspart 30 and for HI30 were 0.03 mM). Mean glucose values decreased with aspart 30 (at 30 min from 5.08 to 4.01 mM, vs from 5.60 to 5.36 mM with HI30), see appendix 1, Table 4. In conclusion, changes in glucose for aspart 30 were higher than for human insulin.

2. The disappearance properties of two different preparations of aspart 30 following use of ^{125}I-labeled biphasic insulin aspart 30 in female crossbred LYYD pigs (Study # 950151):

Methods: After manufacturing of biphasic insulin aspart 30 the crystals are long and thin (REF form), but after few days in the stress test (the rotation test) the crystals are short and broad (ROT form). The disappearance properties of these two preparations were examined in pigs (n=7-8/dose) in a single dose crossover study. 12 U was injected 5 mm into subcutis, of the side of the neck. In this study PK and glucose lowering effects were also examined. Insulin aspart was analyzed using human insulin ——. This formulation of aspart 30 differed from the final clinical formulation used in phase II/III studies, as it contained —— instead of mannitol, and also had higher content of sodium phosphate ——

Results: The radioactivity from two preparations disappeared to the same extent (T 75% was 2.13 and 1.80 hrs for REF and ROT, T 50% was 7.64 and 7.83 hrs

respectively). Both aspart 30 preparations (REF vs ROT) did not show significant differences in the rate of elimination. Glucose values (at 30 min 4.18 vs 4.24 mM) and PK parameters (Cmax 491 vs 464 pM, Tmax 60 vs 48 min, AUC 38.5 vs 27.7 nM/min, T1/2 44 vs 20 min) were also comparable. Thus the size or shape of crystals did not change the blood glucose profiles.

In another study where disappearance of labeled aspart 30/70 (batch # 94016) was compared to human penmix 30/70 (study # 950016), the initial disappearance of aspart 30 was quicker (T 75% 1.76 hrs vs 2.06 hrs, p=0.009), but in the later phase it was similar to human insulin 30 (T 50% 5.13 vs 4.78 hrs, p=0.35), suggesting that it had quick initial course and later same protracted NPH course as human insulin NPH.

3. **The pH comparisons at 7.2 and 7.4 with aspart 30 vs PenMix human insulin 30 (HI 30) in female pigs (Study # 970010):**

Methods: The study objective was to determine the pH limits of aspart 30. Two studies were conducted, using a crossover design. In the first study, female pigs (n=8) were given 0.15 U/kg (SC) of aspart 30 pH 7.2, pH 7.4 or HI 30. The animals were fasted for the first 6 hrs and blood was collected for up to 24 hrs. Insulin aspart was analyzed using [redacted] method for porcine plasma [redacted]. Human insulin was analyzed using [redacted] assay, which also assays endogenous porcine insulin. In the second study, fasted female pigs (n=8) were given 0.15 U/kg (SC) of aspart 30 pH 7.2, or pH 7.6. The blood was collected for up to 24 hrs.

Results: In the first study, the two-pH versions of the biphasic aspart 30 showed no significant differences in the mean glucose levels (at 30 min the values were 3.86 and 4.31 mM for pH 7.2 and 7.4 versions respectively), but both significantly differed from HI 30. The pH 7.2 differed significantly from HI 30 during 20-105 min, and the pH 7.4 differed significantly from HI 30 during 50-90 min. The T1/2 of HI 30 was higher than for two aspart 30 preparations (174 vs 82-93 min), see appendix 1, Table 2. The AUC values for aspart 30 vs HI 30 did not make sense as endogenous porcine insulin was present in the HI 30 assay. Thus, pH change from 7.2 to 7.4 did not alter the PD of the aspart 30. In the second study, the sponsor states that no significant differences were noted with the two pH preparations. T1/2 was equivalent (91 and 105 min) with 7.2 and 7.6 preparations. However, the Cmax (459 and 251 pM, p>0.05, NS) and Tmax (30 and 45 min, P=0.58) values were different with the two above formulations. The pH in the finished clinical product has been set to 7.20-7.44.

In another study where disappearance of labeled aspart 30/70 (batch # Q95008) was compared to human penmix 30/70 (study # 950332a) at pH 6.5 in 6 pigs, the disappearance of aspart 30 was not different from human insulin 30 (T 75% 2.78 hrs vs 1.98 hrs for human insulin, p=0.22).

4. **Comparative blood glucose lowering effects of aspart 30 (0.2 U/kg, SC), pH 7.1 and 7.4 formulation with that of human Penmix 30/70 (study # 960267)**: In female pigs (n=8, in a cross-over study), the pH 7.1 preparation showed a smaller effect on glucose than the pH 7.4 formulation. No significant differences were noted between the 7.1 formulation and human insulin. Average glucose changes at 30 min were –0.78, -1.44 and -0.25 mmol with pH 7.1, 7.4 and human insulin respectively.

5. **Comparative blood glucose lowering effects of biphasic aspart 30 (BIASP) in different ratios (0.15 U/kg, SC), with that of soluble insulin aspart ISAP (study # 980391):** In female pigs (n=8), the BIASP formulated in the ratios as ~ 30, 50, 70, and IASP were examined in a cross-over design for their glucose lowering effects at doses of 0.15 U/kg, given as single SC injections. The specific insulin aspart —— method was used to assay the drug levels. The results by ANOVA analysis indicated that a single SC injection of BIASP 30 caused a more pronounced decrease in plasma glucose than BIASP——————————————————, see appendix 1, Table 3. Sponsor states that these changes in glucose were noted at 20-105 min for BIASP 30, compared to at 150 min for BIASP~ Figure 1 (appendix 1) shows that there is a significant difference in glucose curves between —— and all other biphasic formulations (in the ratios of 30, 50, 70, 100), but no differences were noted between the ratios of 30, 50, 70 or 100 at 0-180 min. The mean plasma Cmax of the drug seemed to increase with increasing the admixture of insulin aspart soluble (from 127, to 181, 242, 454, and 731 pM). Mean residence time (MRT) decreased from 526 min to 92 min with increasing admixture of insulin aspart. The plasma elimination half life (T 1/2) did not quite fit the linear pattern (178, 84, 113, 65 and 44 minutes at ratios of ~ 30, 50, 70 and 100 respectively), but was generally shortest for the aspart BIASP 100. The Tmax values were fairly constant (—— min) with the latest tmax occurring for the BIASP-100 (45 min). Aspart 30 showed higher plasma conc (181 vs 127 pM) with shorter plasma half life (84 vs 178 min) than BIASP~ However no significant differences were noted in T ½ between BIASP 30 vs BIASP 50 or 70 (appendix 1, Table 3). Sponsor states that the overall bioavailabilty of biphasic insulin aspart appeared to be similar for all preparations as demonstated by AUC values.

Single dose pharmacokinetic study in rats(study # 980238):
Single dose PK studies in 36 male and 36 female rats were conducted with doses of 4 and 8 U/kg of aspart 30. Blood samples were analyzed for the drug using —— method. Sponsor states that Cmax (males 15.5 and 20.5 nM at actual doses of 3.1 and 6.1 U/kg, females 17.6 and 31.8 nM at actual doses of 4.3 and 8.3 U/kg respectively) and AUC values (males 450 and 873 nM/min, females 679 and 1516 nM/min) generally increased with the dose. There were no

gender differences in plasma conc with the aspart as previously demonstrated in 1-year tox studies.

TOXICOLOGY (Single dose toxicology)

Acute Toxicity

Effects of Aspart 30 After Single Subcutaneous Injection in SD Rats (Study No. 960200)
Sponsor's ID Study # 960200
Amendment #, Vol #, and page #: Initial NDA, volume 11, page 274
Conducting laboratory: Novo Nordisk, Denmark.
Date of study initiation: 04/03/1997
GLP compliance: Yes
QA Report: Yes (X) No ( ).

Methods:
Dosing: SC
Species: Sprague-Dawley (MOL:SPRD) rats
#/sex/group or time point: 5/sex/group
Age: ≈ 4-5 weeks of age
weight: 86-105 g.
Dosage groups in administered units: The objective was to determine the acute toxicity of aged (3 months at 37^{0}C) and freshly prepared aspart 30 in rats. Eight groups of SD rats (5/sex/group) were administered a single dose of either vehicle (0.15% phenol, 0.172% M-cresol, mannitol 0.36%, NaCl 10nM, containing 0.003% protamine sulfate) or aspart 30 aged drug (Batch No. C96020/3AE/37, TKS #105) subcutaneously at doses of 62.5, 250, 500, 1000, 2000 U/kg, or aspart 30 fresh drug (batch No. C96020, TKS #104) at same doses.

Route, form, volume, and infusion rate: Single subcutaneous injections (SC).
Drug, lot #: Aspart 30 aged drug (Batch No. C96020/3AE/37, TKS #105), aspart 30 fresh drug (batch No. C96020, TKS #104), 100 U/ml, dosed at 10-20 ml/kg.

Formulation/vehicle: Vehicle was 0.15% phenol, 0.172% M-cresol, mannitol 0.36%, sodium phosphate 0.125%, NaCl 10nM, protamine sulfate 0.003%. Note that the vehicle in approved aspart (in NDA 20-986) contained 0.15% phenol, 0.172% M-cresol and 1.60 % glycerol.

Times at which Observations are made:
Clinical signs: 0-30 min and 2 hrs post dosing and then daily up to 2 weeks
Body weights: **Every** 3-4 days
Gross pathology: At sacrifice.

Results:
Mortality and Clinical Signs: There were no deaths in any of the groups throughout the observation period. All animals at 2000 U/kg and some at 1000 U/kg had a decreased motor activity and most animals at this dose showed piloerection. In the high dose groups slight ptosis was noted. Most of these signs were seen at 0-30 min after dosing.

Body Weight and body Weight gain: There were no inter-group differences in body weight gain for 2 week-period.

Gross pathology: No treatment related effects were observed.

Conclusion: The NOAEL was 500 U/kg. The lethal dose level was greater than 2000 U/kg in both sexes of SD rats. No differences in acute toxicity were observed between the aged and freshly prepared drug.

SPECIAL TOXICOLOGY

Local toxicity

Local toxic effects of aspart 30, two and five days after subcutaneous Injection in Pigs (Study No. 960058)

Methods: The objective was to determine the local reaction at the injection site, at 2 and 5 days after SC injections of 3 different formulations of 100 U/ml of aspart 30 in pigs (n=4/SC injection), vs that of human insulin 30 PenMix HM (HI30). Also local reaction of protaphane HM (ge), corresponding three different media for aspart 30, and 0.9% saline were examined. The three formulation of aspart 30 were as follows:

Formulation 1 (batch # H96005) contained — mM mannitol + — mM NaCl + mM phosphate.
Formulation 2 (batch # H96007) contained ———— + — mM NaCl + mM phosphate.
Formulation 3 (batch # H96010) contained ———— + — mM phosphate. 200 µl of different formulations was given subcutaneously.

Results:
The three preparations of the drug produced identical changes, such as slight to moderate SC mixed inflammatory cell infiltration, and all 3 produced clusters of crystals at one or two injection sites. Protaphane HM (ge) and PenMix HM (ge) produced similar but slightly more extensive changes. These changes were basically similar 2 and 5 days after injection. However on day 5, with formulation 2 and 3, clusters of intermediate to large crystals containing foreign giant cells were seen. In contrast, the three different vehicles or saline produced no local

changes. The results showed that 3 formulations of aspart 30 produced identical local changes, and were not significantly different from the local changes produced by human insulin 30, or protaphane HM in pigs.

Local toxic effects of aspart-30, two, five and 21 days after subcutaneous injection in pigs (Study No. 960059):

Methods: The objective was to determine the local reaction at the injection site, after 2, 5 and 21 days of SC injections of one formulation of 100 U/ml aspart 30 in pigs (n=8/group, batch # H96005, 200 µl injections). The effects were compared to HI 30, protaphane HM, corresponding media for the aspart 30, and 0.9% saline. The formulation of aspart 30 contained — mM mannitol + ~ mM NaCl + ~ mM phosphate.

Results:
The formulation of aspart 30, containing — mM mannitol produced identical local changes on day 2, 5 and 21 as seen in a previous study, i.e. mixed inflammatory cell infiltration at different levels, and clusters of crystals at 1-5 injection sites. These were not significantly different from the local changes produced by HI 30, or protaphane HM in pigs.

Immunogenicity
Immunogenicity of fresh and aged biphasic insulin aspart (BIASP-30) after subcutaneous injection in rabbits (Study No. 980002A, 980002B and 940479)

Methods: After long time storage of insulin, small amounts of insulin byproducts emerge which might result in an increase in insulin antibodies. The objective was to compare the immune responses of 20 IU of aged (3 months at 37^{0}C, batch # H98004, containing 3.4% of insulin aspart related products) and freshly prepared (batch # H98003, containing 21.1% of insulin aspart related products) aspart 30 in rabbits. Protaphane MC porcine (low immunogenic) and Ultralente MC bovine (high immunogenic) insulins were also evaluated. Three studies were carried out. In the first study, five groups of rabbits (5/sex/group) were immunized twice a week for 99 days each with the 1 ml of Freunds incomplete adjuvant emulsion containing the drug, bovine or porcine insulin MC. In the second study, rabbits were immunized twice a week for 99 days similarly as in study 1, each with the fresh and aged BHI 30 (Penmix 30, containing 1.2% of fresh and 6.1% aged insulin related products), as well as with bovine and porcine insulin MC. In the third study, rabbits were immunized with the BIASP ——————————————, and with bovine and porcine insulin MC. Serum from rabbits was analyzed for specific insulin antibody response before treatment and every 14 days. Rabbits were chosen, because rabbit insulin differs from human insulin by only one amino acid (B30 Ser). Data were analyzed by Kruskall-Wallis test

Results:
The bovine insulin MC produced significant immune response, as did both preparations of aspart 30. Biphasic aspart 30 produced significantly higher immune responses than both fresh and aged human insulin 30 (BHI 30). With aspart 30 fresh and aged 7/10 and 9/10 rabbits had antibody responses, while with BHI 30 fresh and aged 3/10 and 3/10 rabbits had antibody responses. With porcine and bovine 5/10 and 10/10 rabbits had immune responses. No significant differences in immune responses between aged and new biphasic aspart 30, or BHI 30 were observed, but significant differences were noted between aspart 30 and porcine insulin MC ($p<0.0012$). Both human and porcine gave relatively low standard immune responses while bovine and aspart 30 gave relatively high standard immune responses.

Rabbits developing antibody responses:

Insulin Product	Fresh	Aged
Aspart 30	7/10	9/10
BHI 30	3/10	3/10
Protaphane MC porcine	5/10	-----
Ultralente MC bovine	10/10	-----

The sponsor explains that the reason why high immune responses in the aspart 30 and bovine insulin were observed are because the amino acid compositions are two changes for insulin aspart and three changes for bovine insulin, compared to rabbit insulin. With human insulin, there is only one change of aa for insulin aspart. The BIASP [redacted] was equally immunogenic to porcine insulin (4/10 vs 4/10 in both groups of rabbits had antibodies), but less immuongenic then bovine insulin (4/10 vs 7/9 in BIASP [redacted] treated rabbits respectively had antibodies). The antibody formation in rabbits with BIASP 30 peaked during the first 1-2 months and declined thereafter.

OVERALL SUMMARY AND EVALUATION:

Introduction: Insulin aspart was approved in June 2000 for the treatment of diabetes mellitus. Insulin Aspart (X14) is an analog of human insulin, in which the amino acid, proline, in position β28, has been replaced by aspartic acid. This modification was designed to decrease the part of the molecule responsible for self-association. Thus, X14 is monomeric, and after injection, is released quickly from the subcutaneous tissue, thereby exerting an earlier onset of effect than human insulin, which has to dissociate from dimers to monomers before absorption from the subcutis. Thus aspart is marketed as a rapid acting insulin. The drug is produced by recombinant DNA technology in the yeast sacharomyces cerevisiae. The host strain is identical to the host strain used for the production of human insulin.

The present submission of aspart 30 ([redacted] 70/30) provides for a new formulation of this product for more rapid onset, than biphasic human insulin 70/30, due to its faster absorption. Aspart 30 contains 30% soluble insulin aspart and 70% insulin aspart protamine crystals. The insulin aspart 30 would provide patients with rapid acting premixed insulin, similar to the human insulin NPH. The primary difference of this product compared to the approved Novolog (insulin aspart) is that of the time-action profile. Given the extensive preclinical work done for the original NDA 20-986, limited preclinical studies have been provided in this NDA. In majority of preclinical studies, the formulation used is the same as that used in the final formulation in the clinical phase II/III studies. However, in some studies, an investigational formulation was used which differed slightly from the final formulation [redacted] The primary concerns with this product are the time-action and effectiveness in the clinical population, as well as the potential for antigenicity. Pivotal studies to determine the activity profile have been done in humans. The toxicity profile of most insulin products relates to the limitations of dosing due to severe hypoglycemia. Therefore, extensive pre-clinical studies of this product were not necessary.

Pharmacology / Pharmacokinetics of aspart 30: The primary pharmacology study with aspart 30 in pigs showed that the time to minimum blood glucose (T_{min}) was shorter for Aspart 30 than for HI 30 (129 vs 176 min, $p<0.05$)), and delta changes in glucose for aspart 30 were higher than for human insulin 30 (-1.05 vs –0.2 mM, $p<0.05$). The change in pH from 7.2 to 7.4 in the aspart 30 formulation did not significantly change the mean glucose levels (at 30 min the values were 3.86 and 4.31 for pH 7.2 and 7.4 formulations respectively). However, pH 7.2 differed significantly from HI 30 during 20-105 min, and the pH 7.4 differed from HI 30 during 50-90 min. In contrast, lowering the pH of the aspart 30 to 7.1 produced smaller changes in the blood glucose levels (-0.78 with pH 7.1 vs –1.44 mmol with pH 7.4). The pH specifications of the finished clinical product have been set to 7.20-7.44. Aspart 30 caused a more pronounced decrease in plasma glucose than [redacted] The changes in glucose were noted at 20-105 min with aspart 30, comared to at 150 min for [redacted]. The overall bioavailabilty of biphasic insulin aspart appeared to be similar for all preparations.

Pharmacokinetic studies in pigs indicated that the plasma half life of two aspart 30 preparations (pH 7.2 and 7.4) was shorter than for HI 30 (82-93 vs 174 min). No significant differences were noted when PK of aspart 30 at pH 7.2 and pH 7.6 were compared (Cmax was 459±310 and 251±186 pM, Tmax was 30 and 45 min, T1/2 was 91 and 105 min with 7.2 and 7.6 preparations respectively) due to large variability. Single dose PK studies in male and female rats with doses of 4 and 8 U/kg of aspart 30 showed general increases with the dose in Cmax (males

would not identify hyperglycemia in patients with Type 2 diabetes. Although the number of patients with Type 1 diabetes was limited, the average glycemic control, as measured by HgbA1c, was mediocre at best so some hyperglycemia would have been expected. There were 9 cases (#4, 26, 33, 73, 102, 152, 169, 323, and 437; mean age 43 years; range 25-71) of flu-like illness for patients treated with X-14 70/30 and 5 (#27, 146, 290, 413, and 501; mean age 61 years; range 41-71) for patients treated with human insulin 70/30 (Vol. 32, p306-7). It is not known whether any of these were presentations of DKA.

13.4.--Allergic Reactions

There were no anaphylactoid reactions during study 038.[1] There were several reactions that may be consistent with allergic reactions:

a--A 42 year old female (#433) developed pruritus on the "neck and skin" four days after starting X-14 70/30. The duration of the pruritus was unclear. The patient was not discontinued.

b—A 26 year old female (#323) developed pruritus 25 days after starting X-14 70/30. The duration of the pruritus was unclear. The patient was not discontinued.

c--A 64 year old male (#883) developed persistent "eruption of the skin on the trunk" 17 days after starting X-14 70/30. The patient was discontinued.

d--A 70 year old male (#73) developed a rash 80 days after starting X-14 70/30. The duration of the rash was unclear. The patient was not discontinued.

e--A 71 year old male (#152) developed a rash 59 days after starting X-14 70/30. The duration of the rash was unclear. The patient was not discontinued.

f—A 69 year old female (#778) developed a persistent "exanthem" 14 days after starting study drug human insulin 70/30. The patient was discontinued. The patient appears to have been on insulin mixtures prior to study 038.

There were no narratives available and the CRF were brief and illegible so that further conclusions about the nature of the events cannot be made-except that increased cross-reacting antibody levels do not appear to be associated with increased risk for rash or systemic allergic reactions. (See samples in appendix 2.)

There was insufficient information to determine whether there was a treatment difference in skin injection site reactions.

[1] There was another report of persistent "allergy" that occurred in a 63 year old female after 83 days of treatment with a still blinded drug in study — safety update, p025.

13.5.--Deaths

There were no deaths in the 12 week controlled trial although two patients died during the extension studies. One patient (#235) from the human insulin 70/30 treatment arm died of disseminated non-Hodgkins lymphoma one day after diagnosis during the extension trial (067). Another patient (#147) treated with X-14 70/30 reportedly died of cardiac failure (Safety update: p12, 20). In the first case, not attribution could be made to the drug product. In the second case, the patient appears to have been ineligible for the study

8/29/00 SU+other major biopharm data (9/5/00 FAX)

1.10. Table of contents

2.--Introduction

The Diabetes Control and Complications Trial (DCCT) established that good glycemic control decreased the risk for long-term diabetic complications in patients with Type 1 diabetes mellitus. Intensive therapy was associated with lower HgbA1c values and better clinical outcomes than conventional therapy. Typically, intensive therapy involves pre-prandial dosing with a more rapid acting insulin in conjunction with a longer acting insulin to provide a basal level of control throughout the day. Four or more injections are required daily. Alternatively, patients utilize subcutaneous insulin infusions delivered by pump. A basal rate is based on the anticipated activity level. Insulin boluses are given to cover food consumption. Additional insulin is given in the event of unexpected hyperglycemia. Conversely, insulin rates/injection doses are reduced in the event of hypoglycemia.

Intensive therapy requires frequent monitoring of blood glucose. Fingerstick sampling is typically performed between four and six times per day. Some patients are unable or unwilling to use intensive therapy because of the number of insulin injections required, the complexities of pump use, and/or the number of glucose fingerstick checks. Unfortunately, tight glycemic control is also associated with increased risk of hypoglycemia. These patients and their physicians may elect to pursue conventional therapy with BID dosing regimens instead. Typically a rapid acting insulin is given in conjunction with a longer acting insulin e.g. NPH, lente, or ultralente at breakfast and with the evening meal. In other words, the rapid acting insulin provides glycemic control for the meal immediately following. The longer acting insulin provides insulin coverage for the mid-day meal, the pre-bedtime snack, and the nocturnal interval. If patients mix their own insulin, the ratio of rapid acting insulin to longer acting insulin can be adjusted for anticipated meal size and physical activity. Pre-mixed insulins have a fixed ratio. This may be perceived as "easier" by patients, and it reduces potential contamination of the short acting insulin vial with protamine, a compound used to delay absorption. Fixed-ratio insulins, however, are less flexible, particularly for patients with erratic schedules. They do not permit easy adjustment for the physiologic needs associated with two meal periods. Adjustment for one meal and exercise period frequently results in hyperglycemia/hypoglycemia with the other meal period. Most patients are unable to achieve tight glycemic control with BID insulin dosing and this is accentuated in patients using fixed-ratio insulins.

The development of pre-mixed insulins incorporating the rapid acting insulin analogue, insulin aspart/X-14/Novolog,

[redacted]

[redacted] Insulin Aspart Protamine (IAP). Protamine was mixed with X-14 to prepare a fixed ratio insulin that would have a more rapid onset of action than 70/30 (70% NPH+30% human regular), one of the currently available fixed dose insulins (although the rapidity of onset may be similar to that of 50% NPH+50% human regular). This insulin could be given immediately before meals (versus 30-45 minutes before meals). [redacted]

The sponsor has presented comparative PK-PD data from 70%IAP+30%X-14 vs 70%NPH+30%human regular insulin and 70%IAP+30%X-14 vs 100% X-14 as well as three month efficacy/safety data from a single trial, study 038, in patients with IDDM or NIDDM. The sponsor did not present comparative data with their other biphasic insulins, nor did they present comparative data distinguishing 70%IAP+30%X-14 from NPH or [redacted] or 50% human insulin NPH+50% regular insulin. Because protamine and insulin analogues are antigenic, sponsors have been encouraged to provide long-term data on the magnitude and clinical significance of such antigenicity. The sponsor provided antibody, insulin dose, glycemic control, and allergic reaction data for the three month trial. Limited interim data from the extension trial, study 067, were presented, but the raw data were not available for review.

3.--Prior Agreements

In lieu of extensive clinical testing, the sponsor was requested to provide:
a--pharmacokinetic and pharmacodynamic studies that would demonstrate that each mixture was distinct from the other X-14 mixtures and from X-14 as well as NPH (or [redacted]

b--labeling that would show the how the X-14 products compared to one another on a pharmacokinetic-pharmacodynamic (PK-PD) basis,
c--labeling that would show how the X-14 products compared to human insulin products on a PK-PD basis, (Head-to-head comparison studies would not be required.)
d—and multi-year studies to assess long-term changes in the levels of cross-reacting antibodies and the effect of these antibodies on the doses of insulin required to maintain comparable levels of glycemic control as measured by HgbA1c.

On 2/19/99 Ms. T. Marion and Dr. McElligott were contacted to discuss the importance of doing studies that would show the PK-PD profile of any X-14 insulin mixture with other insulins in the X-14 family and with its counterpart in the human insulin family. [redacted], was also discussed. The sponsor was requested to provide data showing the distinctiveness of X-14 70/30 from other X-14 products by May, 2000 for consideration in this review cycle. The sponsor submitted interim data from two arms of a four-arm study (1086). Reformatting of the data were requested. The CD-ROMs received were unreadable. Replacement data had not been received at the time of this review, but were added 9/8/00.

4.--Objectives

The sponsor has sought to show that:
a--the PK-PD profile of X-14 70/30 mix is distinct from human insulin 70/30 and
b--there were no major differences in glycemic control for patients treated with X-14 mixtures vs human insulin mixtures.

5.--CANDA

There was no CANDA submission. Additional data were provided on EXCEL spread sheets. (The data on the spread sheets were not corrected for incorrect treatment administration after randomization.)

6.—Financial disclosure

Dr. Anders Lindholm has indicated that there are no financial interests to disclose. At the time of this review, such disclosure was not provided for the subsequently submitted cross-over study (#1086).

7.—Pediatric waiver

The sponsor was previously granted a pediatric waiver because most pediatric patients with diabetes, especially those who are prepubescent, are Type 1 patients. Fixed ratio and BID dosing cannot provide the tight control needed to avoid the long-term complications of diabetes. Even in post-pubertal patients with diabetes primarily linked to childhood obesity, tight control is likely to be important because of the expected long duration of disease. Such BID dosing regimens with fixed ratios are unlikely to provide tight control and minimize insulin-hunger that could foster progressive obesity.

8--Chemistry issues

Recombinant X-14 insulin analogue is produced in Saccharomyces cerevisiae using DNA technology is similar to that employed by the sponsor for the production of other insulin products. Insulin Aspart Protamine is produced by adding protamine (approximately 0.33 mg/ml) to an X-14 — insulin, not by [redacted] The specifications for the biphasic 70/30 mix permit the soluble component to range from [redacted] % at expiry. Mannitol was added to a final concentration of 36.4 mg/ml. Sodium chloride was added to a final concentration of 0.58 mg/ml. Zinc [redacted], was added to a final concentration of 32.7 ug/ml. Phenol and meta-cresol concentrations were 1.50 mg/ml and 1.72 mg/ml respectively. The suspension is then [redacted] to a final pH of 7.20 to 7.44. The pre-filled — (3 ml), cartridges (3 ml), and vials (10 ml) are filled with pre-mixed suspensions prepared in this way. The sponsor intends to eliminate [redacted] from its closures to reduce potential allergic responses. [redacted] Stability studies were not complete at the time of NDA submission.

The formulation was changed during development (Table 1). [redacted]

Table 1
Insulin Formulations Used in Studies

Ingredients	Composition 1 Phase I clinical trial: 031 (Formulation manufactured until [redacted]	Composition 2 Phase I clinical trial: 033 (Formulation manufactured until [redacted]	Composition 3 Phase I-III clinical trials: ?032, 038, 046, 1086 (Formulation manufactured until [redacted]
insulin aspart	[redacted] (100 U/ml)	[redacted] (100 U/ml)	[redacted] (100 U/ml)
[redacted]	[redacted]	[redacted]	[redacted]
Mannitol	[redacted]	[redacted]	[redacted] (36.4 mg/ml)
Phenol	[redacted] (1.50 mg/ml)	[redacted] (1.50 mg/ml)	[redacted] (1.50 mg/ml)
m-cresol	[redacted] (1.72 mg/ml)	[redacted] (1.72 mg/ml)	[redacted] (1.72 mg/ml)
zinc [redacted]	32.7 ug/ml	32.7 ug/ml	32.7 ug/ml
NaCl	[redacted]	[redacted] (0.58 mg/ml)	[redacted] (0.58 mg/ml)
disodium hydrogen phosphate, dihydrate	[redacted]	[redacted] (1.25 mg.ml)	[redacted] (1.25 mg.ml)
protamine sulphate	~0.33 mg/ml	~0.33 mg/ml	~0.33 mg/ml
PH	7.3	7.3	7.3

9.--Pre-clinical Issues

The sponsor conducted a single-dose, placebo controlled, toxicology study in 80 rats dosed with up to 2000 U/kg of aged and fresh X-14 70/30. Human insulin 70/30 was not used as a comparator. Reportedly there was decreased motor activity and piloerection in animals from the higher dose groups.

Local toxicity studies using the preparations used in the phase 3 studies were not conducted.

Immunogenicity studies conducted in rabbits suggest that X-14 and NPH are more antigenic than human insulin70/30 mixture (Table 2).

Table 2
Immunologic Responses in Rabbits Given Various Insulin Preparations

	X-14 70/30 (fresh)	X-14 70/30 (old)	human insulin 70/30 (fresh)	human insulin 70/30 (old)	NPH	ultralente
# w/o detectable immunogenic response	3	1	7	7	5	0*

#=number There were 5/group/sex
w/o=without
*There was one rabbit death in the study; it occurred in the ultralente group.

The sponsor conducted a single-dose, PK study in rats; n=36 male, n=36 female. The low doses of X-14 70/30 were 4.3 U/kg and 3.1 U/kg respectively in females and males. The

high doses were 8.3 U/kg and 6.1 U/kg in females and males respectively. The sponsor reported linear kinetics (p188).

The sponsor conducted a single-dose, cross-over, PK-PD study in fasted (until 6 hr post dosing), non-diabetic pigs using 0.15 U/kg of human regular insulin and X-14 70/30 pH 7.2 and X-14 70/30 pH 7.4, n=8. (Pig skin is relatively similar to human skin so pigs are good models to assess absorption profiles.) Reportedly the X-14 70/30 mixtures lowered insulin more promptly than human regular insulin, and the pH did not alter the PK results significantly for the two X-14 70/30 mixtures. The insulin t1/2 values were 174 minutes, 93 minutes, and 82 minutes for human insulin 70/30, X-14 70/30 (pH 7.4), and X-14 70/30 (pH 7.2) respectively. Reportedly the X-14 70/30 mixtures lowered glucose more promptly than human regular insulin, and the pH did not alter the PD results significantly for the two X-14 70/30 mixtures. Reportedly the glucose levels with the pH 7.2 version differed statistically from the glucose levels with regular insulin 20 to 105 minutes post dosing, and the glucose levels with the pH 7.4 preparation differed from regular insulin 50 to 90 minutes post dosing. The glucose profiles for the two X-14 compounds were not appreciably different. This was followed by another cross-over study in eight pigs using a single dose of 0.15 U/kg using two formulation of X-14 70/30, pH 7.2 vs 7.6. There were some differences. Cmax was higher (459 vs 251 pM), tmax shorter (30 vs 45 max), and t1/2 (91 vs 105 min) shorter for the formulation with the pH of 7.2 than the pH of 7.6. This was followed by yet another PD study in eight, non-diabetic pigs using 0.2 U/kg of human insulin 70/30 and X-14 70/30 pH 7.1 and X-14 70/30 pH 7.4. The mean glucose lowering 30 minutes post injection was -14 mg/dl, -26 mg/dl, and +4.5 mg/dl for X-14 70/30 pH 7.1 and X-14 70/30 pH 7.4 respectively. Although the interpretation of both the PK and PD data from these studies may be limited by the secretion of endogenous insulin, the sponsor concluded that the pH range for the insulin product should be limited to 7.2 to 7.4.

A 5-way cross-over PK-PD study was conducted in eight pigs dosed using 0.15 U/kg of X-14, X-14 30/70, X-14 50/50, X-14 70/30, and [redacted] The data suggest that there are glucodynamic differences between [redacted] and the X-14 mixtures, as well as X-14. The glucodynamic differences between the mixtures and the differences from X-14, however, are less clear (p187, Figure 1).

10.--Pharmacokinetic-Pharmacodynamic Issues

10.1. Formulation changes

The formulation was changed during development (Table 1). [redacted] PK-PD bridging studies comparing the various formulations were not conducted.

10.2. Protamine changes

Studies to assess the inter-changeability of protamine as long and thin crystals and protamine as short and broad crystals were done (Study 032). The study showed that the crystal forms are interchangeable.

10.3. Pre-mixing vs self-mixing

The sponsor did not do any studies to show that self-mixed combinations of X-14 70/30 was pharmacokinetically similar to the same insulin combination when given as a pre-mixture.

10.4. Comparative studies

10.4.1. X-14 70/30 vs human insulin 70/30

In a single-dose, crossover study in fasted, normal volunteers (031; n=23, formulation #1) dosed with X-14 70/30 and human insulin 70/30, the ratio of the respective AUC $_{\text{insulin (0-90 min)}}$ values was 1.86; $p<0.0001$, the ratio of the respective C_{max} values was 1.51; $p<0.001$, and the difference of the respective t_{max} values was –60.0; $p<0.001$. In a single-dose crossover clamp study in fasted normal volunteers (033; n=32, formulation #2) dosed with X-14 70/30 and human insulin 70/30, the ratio of the respective AUC $_{\text{insulin (0-90 min)}}$ values was 2.24; $p<0.0001$, the ratio of the respective C_{max} values was 2.02; $p<0.001$, and the difference of the respective t_{max} values was –95.0; $p<0.001$ (Tables 3-8). Between-product differences exceeded 20%--suggesting that the PK profiles of these two insulin preparations from different insulin families could be distinguished from one another when given as a single injection.

10.4.2. X-14 70/30 vs X-14

In a single-dose, 4-arm crossover clamp study in fasted normal volunteers (study 1086; 34 received X-14 70/30, 33 received X-14; formulation #3) dosed with X-14 70/30 and X-14, the ratio of the respective AUC $_{\text{insulin (0-120 min)}}$ values was 0.409 ($p<0.001$), the ratio of the respective C_{max} values was 0.454 ($p<0.001$), and the difference of the respective t_{max} values was 0.13 hours ($p=0.295$). Between-product differences exceeded 20% (Tables 3, 4, 9-12).

Table 3

Comparisons of Pharmacokinetic Parameter Ratios of Insulins Using Log Transformed Data (Data from Dr. Sun)

Pharmacokinetic Parameter	Study	Insulin Pair	Mean Ratio	90% Confidence Interval
AUC-insulin(0-t)	031	X-14 70/30 vs HI 70/30	1.048	0.968—1.135
	033	X-14 70/30 vs HI 70/30	1.158	1.08—1.24
	1086	X-14 70/30 vs X-14	0.58	0.546—0.630
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA
AUC-insulin(0-6 hr)	031	X-14 70/30 vs HI 70/30	1.231	1.144—1.325
	033	X-14 70/30 vs HI 70/30	1.608	1.468—1.760
	1086	X-14 70/30 vs X-14	0.485	0.446—0.526
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA
Cmax-insulin	031	X-14 70/30 vs HI 70/30	1.512	1.375—1.662
	033	X-14 70/30 vs HI 70/30	2.02	1.798—2.270
	1086	X-14 70/30 vs X-14	0.38	0.336—0.433
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA

AUC=area-under-the-curve

HI=human insulin

NA=not available

Table 4

Comparisons of Pharmacodynamic Parameter Ratios of Insulins Using Log Transformed Data (Data from Dr. Sun)

Pharmacodynamic Parameter	Study	Insulin Pair	Mean Ratio	90% Confidence Interval
Rmax	033	X-14 70/30 vs HI 70/30	1.197	1.125—1.274
	1086	X-14 70/30 vs X-14	0.763	0.719—0.813
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA
AUC-glucose (0-t)	033	X-14 70/30 vs HI 70/30	0.975	0.902—1.055
	1086	X-14 70/30 vs X-14	0.925	0.869—0.990
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA
AUC-glucose (0-6 hr)	033	X-14 70/30 vs HI 70/30	1.219	1.140—1.305
	1086	X-14 70/30 vs X-14	0.826	0.780—0.877
	1086	X-14 70/30 vs X-14 50/50	NA	NA
	NA	X-14 70/30 vs —	NA	NA

Rmax=maximal glucose utilization or maximal glucose infusion rate
AUC=area-under-the-curve
HI=human insulin

Table 5

Mean (CV) Times to Partial and Total Insulin AUC Values for X-14 70/30 and Human Insulin 70/30*

Parameter T-AUC_{hr}	X-14 70/30		Human Insulin 70/30		Ratio	
	Study 031	Study 033	Study 031	Study 033	Study 031	Study 033
25%	2.41 (22)	2.78 (21)	3.32 (14)	4.58 (16)	0.73	0.61
50%	5.45 (21)	7.16 (22)	6.80 (14)	9.85 (15)	0.81	0.73
75%	11.02 (16)	14.70 (11)	12.38 (15)	15.92 (9)	0.91	0.93
100%	23.83 (4)	24.00 (0)	24.00 (0)	24.00 (0)	0.99	1.00

*T-AUC-100% are the times to reach the given % of the total AUC for each formulation

Table 6

Mean (CV) Times to Partial and Total Insulin AUC Values for X-14 70/30 and Human Insulin 70/30 as Compared to Human Insulin 70/30*

Parameter T-AUC_{hr}	X-14 70/30		Human Insulin 70/30		Ratio	
	Study 031	Study 033	Study 031	Study 033	Study 031	Study 033
25%	2.34 (23)	2.48 (30)	3.32 (14)	4.58 (16)	0.70	0.55
50%	5.39 (34)	6.08 (38)	6.86 (14)	9.85 (15)	0.79	0.62
75%	11.15 (40)	11.85 (36)	12.38 (15)	15.92 (9)	0.92	0.74
100%	15.81 (32)	15.66 (24)	24.00 (0)	24.00 (0)	0.66	0.65

*T-AUC-100% for X-14 70/30 are the times when the same respective AUC values were achieved for human insulin .

Table 7

Mean (CV) Times to Partial and Total Glucose AUC Values for X-14 70/30 and Human Insulin 70/30*: Study 033

Parameter $T\text{-}AUC_{hr}$	X-14 70/30	Human Insulin 70/30	Ratio
25%	3.18 (15)	4.13 (17)	0.79
50%	6.48 (16)	8.33 (14)	0.79
75%	12.90 (14)	14.30 (12)	0.91
100%	23.98 (0)	24.00 (0)	1.00

*T-AUC-100% are the times to reach the given % of the total AUC for each formulation

Table 8

Mean (CV) Times to Partial and Total Glucose AUC Values for X-14 70/30 and Human Insulin 70/30 as Compared to Human Insulin 70/30*: Study 033

Parameter $T\text{-}AUC_{hr}$	X-14 70/30	Human Insulin 70/30	Ratio
25%	3.31 (21)	4.13 (17)	0.81
50%	7.30 (36)	8.33 (14)	0.87
75%	12.70 (34)	14.30 (12)	0.91
100%	17.00 (25)	24.00 (0)	0.71

*T-AUC-100% for X-14 70/30 are the times when the same respective AUC values were achieved for human insulin .

Table 9

Mean (CV) Times to Partial and Total Insulin AUC Values for X-14 70/30 and X-14*: Study 1086

Parameter T-AUC	X-14	X-14 70/30	Ratio
25%	1.08 (24)	1.44 (20)	0.76
50%	1.74 (22)	2.61 (21)	0.68
75%	2.62 (24)	4.95 (20)	0.54
100%	10.00 (0)	24.00 (0)	0.42

*T-AUC-100% are the times to reach the given % of the total AUC for each formulation

Table 10

Mean (CV) Times to Partial and Total Insulin AUC Values for X-14 70/30 and X-14 Insulin as Compared to X-14 70/30*: Study 1086

Parameter $T\text{-}AUC_{hr}$	X-14	X-14 70/30	Ratio
25%	0.84 (27)	1.44 (20)	0.59
50%	1.27 (30)	2.61 (21)	0.49
75%	1.66 (35)	4.95 (20)	0.34
100%	2.27 (48)	24.00 (0)	0.09

*T-AUC-100% for X-14 70/30 are the times when the same respective AUC values were achieved for X-14 .

Table 11
Mean (CV) Times to Partial and Total Glucose AUC Values for X-14 70/30 and X-14*: Study 1086

Parameter T-AUC_{hr}	X-14	X-14 70/30	Ratio
25%	1.91 (15)	2.17 (17)	0.89
50%	3.18 (16)	3.66 (14)	0.87
75%	4.78 (17)	5.70 (14)	0.85
100%	10.00 (0)	10.00 (0)	1.00

*T-AUC-100% are the times to reach the given % of the total AUC for each formulation

Table 12
Mean (CV) Times to Partial and Total Glucose AUC Values for X-14 70/30 and X-14 as Compared to X-14*: Study 1086

Parameter T-AUC_{hr}	X-14	X-14 70/30	Ratio
25%	1.84 (24)	2.17 (17)	0.85
50%	3.07 (26)	3.66 (14)	0.84
75%	4.52 (29)	5.65 (13)	0.80
100%	5.52 (29)	10.00 (0)	0.55

*T-AUC-100% for X-14 70/30 are the times when the same respective AUC values were achieved for X-14 .

10.4.3. Other comparators
-The sponsor did not compare X-14 70/30 with the most appropriate human insulin mixture 50/50. The latter is known to have a more rapid rate of absorption and onset of action than human insulin 70/30.
-The sponsor did not provide data comparing X-14 with neighboring members of the X-14 family: 50/50 and [redacted] was one of the treatment arms in the 4-arm crossover study with X-14 and X-14 70/30.
-The sponsor did not compare X-14 70/30 with the most appropriate basal insulin, NPH.

11.--Study design for clinical trials
11.1.--General
The sponsor conducted one three-month, parallel, open-label active control, 1:1 randomization clinical trial with the mixture proposed for registration (formulation #3): 038 (Table 13). The study were conducted outside the U.S (Table 14). Patients with both Type 1 and 2 diabetes were enrolled. Diabetic patients over the age of 17 were enrolled. All patients were to have had experience with insulin therapy. (See inclusion criteria.) Patients were then randomized to three months of treatment with an X-14 or human insulin mixture (Table 13). Injections of human insulin 70/30 were to be given 30 minutes before breakfast and supper; injections of X-14 70/30 were to be given 15 minutes or less before breakfast and supper. Injections could be given in the thigh or abdomen-per local custom. (Results were not to be stratified by injection site although it is known that PK-PD responses vary by injection site.) Patients performed home glucose

monitoring. Insulin doses were titrated to maximize glycemic control and minimize hypoglycemia. Patients were then eligible to enter extension trials 067. Longitudinal cross-reacting insulin antibody data are being collected in — extension trials.

Table 13
Design Features of the Clinical Study

Study	Insulin Type	Dosing	Study Type	Tx Arm Duration	Blinding	Glucose Measure	
038	X-14 70/30 vs human insulin 70/30	BID	parallel	3 months	no	HgbA1c	8-point glucometer profile

Tx=treatment

Table 14
Other Study Features

Study	# Investigators	# Countries	Conducted in U.S.	# Randomized Patients/Investigator
038	36*	4**	No	8.17*

*does not include 3 investigators who did not enroll any patients
**does not include an investigator from Switzerland who did not enroll any patients. The other countries include Austria, Germany, Ireland, and United Kingdom.

11.2.--Patient Selection Criteria
11.2.1.--Inclusion Criteria
Aged ≥18 years (except in Austria ≥19 years)
Diabetes mellitus-Type 1 or Type 2 for ≥24 months
Treatment with BID insulin for ≥12 months (The UK required patients to be using Novo mixes *a priori*.)
HgbA1c ≤11%
BMI ≤35 kg/m^2
11.2.2.--Exclusion Criteria
Insulin allergy
Profound insulin resistance: insulin dose ≥1.4 U/kg/d
Inability to do glucose monitoring
Class 3 or 4 cardiac disease or unstable angina or myocardial within the last year
Renal disease (creatinine ≥1.7 mg/dl)
Active proliferative retinopathy
Liver disease (ALT ≥2x ULN [50 IU/l], alk phos ≥2x ULN [144 IU/l])
History of pancreatitis
Pregnancy or risk of pregnancy or lactation
Use of oral anti-diabetic agents-- within 30 days of entry
Use of systemic steroids at the time of entry
Severe recurrent hypoglycemia (There were no established criteria.)

Did not exclude patients:
--at high risk of requiring systemic steroids
--using beta blockers
--who had been exposed previously to X-14
--with adrenal insufficiency
--autonomic neuropathy

11.3.—Patient Characteristics-Special Populations
35% of exposed patients were patients with Type 1 diabetes. The mean duration of diabetes was 15.3 years. 54% of exposed patients were male. The mean age for exposed patients was 56.6 years and is consistent with the study inclusion of patients with Type 2 diabetes. 1% of patients were non-Caucasian; these three patients were randomized to human regular insulin for treatment. The mean BMI was 27.4. 20% of the exposed population were smokers. The values of the important safety and efficacy parameters were similar for the treatment groups at baseline (Table 15).

Table 15
Mean Intent-to-treat Values for HgbA1c, Insulin Doses, and Cross-reacting Antibodies

Study 038 Treatment Group	HgbA1c (%)		Total Daily Dose (U/kg)		Cross-Reacting Antibodies+ (% Binding)	
	Baseline *(n)*	Baseline-with values for ITT *(n)*	Baseline (n)	Baseline-with values for ITT (n)	Baseline (n)	Baseline-with values for ITT (n)
-IDDM	8.39 *48*	8.46 *46*	0.622 *49*	0.627 *47*	13.06 *49*	12.99 *46*
HR-NIDDM	8.18 *102*	8.18 *96*	0.580 *101*	0.579 *96*	8.73 *102*	9.12 *97*
X14-IDDM	8.38 *55*	8.41 *49*	0.631 *53*	0.640 *48*	11.06 *55*	11.49 *49*
X14-NIDDM	8.07 *84*	8.07 *81*	0.561 *85*	0.562 *83*	9.74 *85*	10.08 *82*

The "baseline" values, but not the "baseline-with values for ITT", include the 3 patients treated with the wrong drug: #26, 83, and 574.
Baseline-with values for ITT refers to randomized patients with baseline values and a subsequent value for intent-to-treat assessment.
ITT=intent-to-treat
+It is not known whether these values include the non-specific antibodies.

No special population groups were studied.

11.4.---Numbers of Patients and Disposition
351 patients were screened. 294 patients were randomized. Three patients randomized to X-14 70/30 did not receive any drug. Two patients randomized to X-14 70/30 actually received human insulin 70/30 and completed the trial (#26 and #83). One patient was treated with human insulin 70/30 until the last month of the trial (#574). 279 (96%) had post-baseline data for intent-to-treat (ITT) analysis. 268 (92%) completed the trial. One patient (#720) completed the trial, but did not have endpoint HgbA1c data. Withdrawals were few and scattered throughout the trial (Table 16). The patterns of withdrawal were

similar for the two insulin products (Table 17). Twelve of the 23 patients who withdrew were NIDDM patients. The most common reasons for withdrawal during the trials was non-compliance. Seven patients were discontinued for adverse events. Two patients were withdrawn for rash.

Table 16

Duration of Patient Exposure to Experimental and Control Drug in Patients with Any Drug Exposure after Randomization

	Duration in Study			
Treatment Arm	</=4 weeks	>4weeks, </=8 weeks	>8 weeks	Completers
X-14 70/30	9	2	131 (129)*	124 (122)*
Human insulin 70/30	5	3	145 (144)*	144 (143)*

*The patients treated with the wrong insulin were included in the group to which they were randomized.

Table 17

Discontinuation of Patients

Reason for Discontinuation	Patients Exposed to Drug in the Controlled Trial				
	# Patients			Duration of Treatment (days) for Each Drop-out (Individuals & Group)	
	X-14 mix	HI mix	Total	X-14 mix	HI mix
Non-compliance	5	3	8	21+9+13+14+13=70	80+35+15=130
Adverse event	4	3	7	8+84+63+32=187	14+2+29=45
Entry Criteria or Protocol Violation	2	2	4	55+28=83	71+51=122
Other	2	1	3	5+21=26	15
Lack of efficacy	1	0	0	15	

X-14=insulin aspart HI=human insulin

11.5.— Drug Exposure in Extension Trials

The sponsor did not supply complete information on the extension trials so these data were not reviewed.

11.6--Study Drug Formulation

Insulin X-14 70/30 has the empirical formula of $C_{256}H_{381}N_{65}O_{79}S_6$ and a molecular weight of 5825.8. Each milliliter of X-14 70/30 contains insulin aspart 100 units, 0.33 mg protamine sulfate, 36.4 mg mannitol, 1.25 mg dibasic sodium phosphate, 1.72 mg m-cresol, 1.5 mg phenol, zinc ——— adjusted to provide 32.7 ug/ml, ———
The pH is adjusted to 7.2—7.44.

11.7.—Dose-Route-Administration

All insulin was to be given as subcutaneous injections twice daily with the doses to be titrated as needed (Table 13). One patient (#501) required more injections per day than was permitted by the protocol Another had higher insulin requirements than permitted

(#245). Both were in the human insulin 70/30 treatment arm and were withdrawn from the study.

11.8.-- Concomitant Medications

Patients using glucorticoids, which can increase insulin resistance and the doses of insulin required to maintain glycemic control, were excluded from ANA/DCD/038,UK. Patients using beta blockers, which can mask the symptoms of hypoglycemia, were not excluded from ANA/DCD/038,UK. Oral antidiabetic agents were excluded from the ANA/DCD/038,UK study, but the period for exclusion, 1 month, was not long enough to exclude their impact on basal HgbA1c values. Patients who had participated in other insulin aspart product studies or who had used commercially available insulin aspart were not specifically excluded. Prior exposure to X-14 could have had an impact on cross-reacting antibody levels.

There were no drug interaction studies-although the sponsor recorded the use of concomitant drugs with some of the hyperglycemic events (Vol.46, p244).

11.9.—Safety Studies and Parameters

Physical exams were conducted at study entry. Patients were to have undergone a retinal exam at or three months prior to screening. There was no specific assessment of diabetic neuropathy. Vital signs and weight measurements were taken at each subsequent visit. The exit physical did not include a formal funduscopic exam. Electrocardiograms were obtained at entry and exit. Routine clinical chemistry, hematologic, and lipid tests were obtained at baseline and at the end of each treatment arm. Patients were to conduct serial home glucose monitoring and to report hypoglycemia.[1] There were no specific criteria for monitoring or assessing hyperglycemia. Urine ketones were to be measured with each visit using ketostix. Anti–insulin antibodies, in particular, cross-reacting insulin antibodies, were assessed at baseline and endpoint.

[1]Hypoglycemia was defined by the sponsor as:
Minor--symptoms consistent with hypoglycemia with or without serum/blood glucose confirmation
Major A—symptoms of hypoglycemia with impaired consciousness that required third party assistance
Major B-- symptoms of hypoglycemia with impaired consciousness that required third party intervention with IV glucose or glucagon

11.10.—Efficacy Variables

HgbA1c values, the parameter of glycemic control accepted by the Division, were obtained at baseline and at endpoint. In addition, unblinded patients were to conduct a home glucose profile with sampling done before meals, 90 minutes after meals, before bedtime, and at 2 A.M. Measures were to be obtained on three days in the week prior to the baseline, 8 week, and 12 week visits. The sponsor assessed the mean glucose, glucose excursion, fasting glucose, and post-prandial glucose with these glucometer readings.

11.11.—Statistical Analysis

Active controls were employed because of the absolute requirement for insulin in Type 1 patients. The controls were human insulin mixtures. The study was open-label to permit

administration of the human regular insulin mixes 30 minutes prior to meals and X-14 mixes within 10 or 15 minutes of meal ingestion. Although the sponsor used a non-inferiority comparison for HgbA1c: H_0:d>0.6%, and the alternative H_1:d<0.6%, rigorous statistical analysis was not undertaken because a) the equivalence of lispro and human regular insulin had been previously established, b) the trials were open-label, and c) the variability due to injection site differences was not controlled.

11.12.—Inspections
Inspections of the clinical sites were not initially requested because the clinical study was not the pivotal study. A cross-over PK-PD study that assesses the differences from neighboring insulins: X-14 50/50 mix and [redacted] or NPH would be the most appropriate study for inspection. In May 2000, the sponsor submitted data from a 4-arm cross-over PK-PD study site in Germany. Because the sponsor provided data only from the X-14 and X-14 70/30 arms and did not have a [redacted] arm or NPH arm, the study was deemed to be inadequate. The request to inspect this site was withdrawn because of these inadequacies.

11.13.—Amendments
September 18, 1997
The X-14 70/30 insulin was to be administered within 10 (not 15) minutes of the beginning of a meal.
January 21, 1998
A German version of the quality of life questionnaire was to be used in Austria, Germany, and Switzerland.
February 4, 1998
In Austria, the trial was restricted to patients at least 19 (not 18) years of age.
November 5, 1997
In Ireland, the trial was restricted to patients who had not received another investigational drug within the last 4 (not 3 months).
January 15, 1998
In the UK, the trial was restricted to patients who had used NovoNordisk mixtures (not other brands and not self-prepared mixtures BID) for at least 12 months. Patients were expected to continue on these NovoNordisk mixtures between baseline and study baseline, the mixtures would not be provided by NovoNordisk during this interval.
April 2, 1998
The list of local trial monitors for Austria, Germany, and Switzerland was modified.
July 7, 1998
The list of investigators for Austria, Germany, and Switzerland was modified.

12.–Efficacy Results
Glycemic control as measured by HgbA1c was less than optimal at baseline (Table 15). Mean values exceeded 8%. Glycemic control did not improve substantially during the clinical trial (Tables 18-20). The maximal decrease in HgbA1c was 0.2%. There were no clinically significant differences between the treatment groups for HgbA1c at endpoint and the change in HgbA1c over the duration of the study whether an intent-to-treat or

completer analysis was performed. There were no gender differences for the change in HgbA1c over the duration of the study for patients with NIDDM (Table 21). The same, however, cannot be said for patients with IDDM who were treated with X-14 70/30. The glucose control in the women deteriorated. The small size of this subgroup may contribute to this deviant observation and limits the detection of any interaction between and gender and glycemic control.

Insulin doses were increased for all treatment groups, but did not account for all of the changes in glycemic control. The dose increase was greater for patients in the X-14 70/30 arms. The difference in dose was 0.07 U/kg/d for patients with IDDM and 0.02 U/kg/d for patients with NIDDM. These differences were statistically significant for the IDDM patients. Additional data support the need for higher doses of X-14 70/30 insulin to achieve comparable changes in glycemic control. Patients with IDDM in the human insulin 70/30 arm had a decrease in HgbA1c of 0.20% with an increase in daily insulin dose of 0.012 U/kg/d: ratio –17.09. Patients with NIDDM in the X-14 70/30 arm had a comparable decrease in HgbA1c of 0.18% with an increase in daily insulin dose of 0.041 U/kg/d: ratio –4.38. Patients with IDDM in the human insulin 70/30 arm had a decrease in HgbA1c of 0.20% with an increase in daily insulin dose of 0.012 U/kg/d: ratio –17.09. Patients with NIDDM in the human insulin 70/30 arm had a decrease in HgbA1c of 0.10% with an increase in daily insulin dose of 0.026 U/kg/d: ratio –3.86. In practical terms, the mean differences in dose increases were approximately 1 to 5 U/day for a 70 kg person receiving X-14 as opposed to human insulin. These values are similar to those observed in the original X-14 NDA.

Table 18

Mean Intent-to-treat Values for HgbA1c and Insulin Doses*

Study 038 Treatment Group	HgbA1c *(%)*				Dose *(U/kg/day)*			
	Baseline- *All**	Baseline- *With at least 1 f/u Value*	Endpoint	Delta	Baseline- *All**	Baseline- *With at least 1 f/u Value*	Endpoint	Delta
X-14 70/30 IDDM (n exposed=53)	8.34	**8.41**	8.42	0.01	0.628	0.638	**.713**	**0.074**
N=	53	49	49	49	53	48	48	48
HI 70/30 IDDM (n exposed=49)	8.39	8.46	8.26	**-0.20**	0.622	0.627	0.639	0.012
N=	49	46	47	46	49	47	47	47
P=	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.14	0.005
X-14 70/30 NIDDM (n exposed=85)	8.07	8.07	**7.92**	**-0.18**	0.561	0.562	0.603	**0.041**
N=	84	81	82	81	85	83	83	83
HI 70/30 NIDDM (n exposed=101)	8.16	8.19	8.08	-0.10	0.578	0.579	0.605	0.026
N=	101	96	96	96	100	96	97	96
P=	N.S.	N.S.	N.S	N.S.	N.S.	N.S.	N.S.	N.S.

*Does not include the 3 patients who were treated with the wrong insulin: #26, 83, and 574.
HI=Human insulin

Table 19

Mean Intent-to-treat Values for HgbA1c, Insulin Doses, and Cross-reacting Antibodies in Patients Who Had Values for All Parameters at Baseline and Study Exit*

Study 038 Treatment Group	HgbA1c (%)			Total Daily Dose (U/kg)			Cross-Reacting Antibodies+ (% Binding)		
	Baseline	Endpoint	Delta	Baseline	Endpoint	Delta	Baseline	Endpoint	Delta
X14-IDDM *n=48*	8.41	8.41	-0.002	0.638	**0.713**	**0.075**	11.71	**26.79**	**15.08**
HI-IDDM *n=45*	8.45	**8.28**	**-0.17**	0.619	0.628	0.009	12.26	12.66	0.40
	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=0.089	P=0.003	P=N.S.	P=0.001	P=5.4x10-6
X14-NIDDM *n=80*	8.08	**7.89**	**-0.20**	0.564	0.606	**0.042**	10.24	**18.98**	**8.74**
HI-NIDDM *n=95*	8.19	8.08	-0.11	0.578	**0.604**	0.026	9.28	9.69	0.41
	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=0.002	P=3.7x10-5

*Does not include the 3 patients who were treated with the wrong insulin: #26, 83, and 574. Analysis showed that exclusion of these patients did not substantively change the analysis.

+It is not known whether these values include non-specific antibodies.

X-14=X-14 70/30 HI=Human insulin 70/30

Table 20

Mean Values for HgbA1c, Insulin Doses, and Cross-reacting Antibodies in Patients Who Had Values For All Parameters at Baseline and 12 weeks-Completers*

·ly 038 ·eatment Group	HgbA1c (%)			Total Daily Dose (U/kg)			Cross-Reacting Antibodies+ (% Binding)		
	Baseline	Endpoint	Delta	Baseline	Endpoint	Delta	Baseline	Endpoint	Delta
X14-IDDM *n=47*	8.42	8.39	-0.03	0.638	**0.713**	**0.075**	5.42	**26.31**	**15.44**
HI-IDDM *n=44*	8.46	**8.27**	**-0.19**	0.618	0.627	0.010	12.18	12.68	0.50
	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=0.094	P=0.004	P=N.S.	P=0.002	P=5.2x10-6
X14-NIDDM *n=76*	8.11	**7.92**	**-0.19**	0.556	0.602	**0.046**	9.48	**18.13**	**8.65**
HI-NIDDM *n=93*	8.17	8.06	-0.11	0.574	**0.601**	0.027	9.33	9.75	0.42
	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=N.S.	P=0.005	P=5.8x10-5

*Does not include the 3 patients who were treated with the wrong insulin: #26, 83, and 574. Analysis showed that exclusion of these patients did not substantively change the analysis.

+It is not known whether these values include non-specific antibodies.

X-14=X-14 70/30 HI=Human insulin 70/30

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Table 21
HgbA1c Results by Gender

Study 038	HgbA1c (%) Baseline	HgbA1c (%) Endpoint	HgbA1c (%) Delta	N=
X-14 70/30 IDDM-male	8.53	8.43	-0.10	31
X-14 70/30 IDDM-female	**8.21**	**8.41**	**+0.20**	18
HI 70/30 IDDM-male	8.35	8.17	-0.16	33
HI 70/30 IDDM-female	8.71	8.47	-0.24	14
X-14 70/30 NIDDM-male	7.95	7.79	-0.18	44
X-14 70/30 NIDDM-female	8.23	8.05	-0.17	39
HI 70/30 NIDDM-male	8.12	8.04	-0.08	46
HI 70/30 NIDDM-female	8.22	8.10	-0.12	51

*Does not include the 3 patients who were treated with the wrong insulin: #26, 83, and 574.

The sponsor collected fasting and post-prandial glucose data (Table 22). The latter included 90 minute post-prandial glucose levels, glucose excursions (the 90 minute post-prandial glucose minus the pre-prandial glucose level), the 90 minute post-prandial areas-under-the-curve (AUC) for glucose, and the mean glucose profiles (a composite of glucometer readings obtained before meals, 90 minutes after meals, at bedtime, and at 2 A.M). The meaning of these glucose parameters remains uncertain. No glucose measurements were made via a laboratory. They are all derived from glucometer readings, which are not known for their precision and accuracy, and these readings are collected by the patients in an unblinded fashion. The fasting glucose readings were higher in patients with IDDM who were on X-14 70/30 than in patients with IDDM who were on X-14 70/30. A similar finding was observed with IDDM patients treated with another short acting insulin analogue and a night-time basal insulin and was presumed to occur because of the shorter duration of the insulin analogue than human regular insulin. Curiously, this finding was not replicated in the patients with NIDDM as it was with the other insulin analogue. Furthermore, the mean glucose profiles did not correlate well with another well validated estimate of mean glucose exposure, HgbA1c. Similarly, none of the r values for other glucose parameters, fasting glucose, 90-minute-post-breakfast glucose, glucose excursion, and AUC $_{\text{breakfast to 90 minutes}}$, exceeded 0.6.—suggesting that these parameters lack clinical significance and/or that the self-collected glucose values were not accurate or representative of the true values for the parameters (Tables 22-24, Figures 1—8).

It should be noted that in a small, two week, cross-over study (046) with serial glucose measurements in13 patients with NIDDM, the maximal post-prandial glucose for breakfast and supper were higher by ~18—54 mg/dl for patients using human insulin 70/30, but that maximal post-lunch glucose differences were higher by ~36 mg/dl for patients using X-14 70/30 insulin (Vol. 1, p286). (See graphic display in appendix 1.) Mean nocturnal glucose measurements exceeded 120 mg/dl and did not vary by treatment arm. These data suggest that there are temporal differences in the profiles of glucose lowering for the two different insulin mixtures, but that no one fixed insulin mixture

provides lower glucose values throughout the entire day. Hence, the limited correlation observed between post-prandial parameters and HgbA1c.

Table 22

Glucose Parameters (Derived from Glucometer Readings) and Their Relationship to HgbA1c

Treatment/ Statistical Parameter	Glucose Parameters					
IDDM, X-14 70/30	HgbA1c (%)	Fasting (mmol/L)	90 Min PP (mmol/L)	Glucose Excursion (mmol/L)	AUC-90 Min (mmolxhr/L)	Mean (mmol/L)
Mean	**~8.4**	**9.75**	**10.25**	**0.47**	**14.93**	**8.88**
R=		0.42	0.12	-0.24	0.29	0.24
N=		*46*	*46*	*45*	*45*	*37*
IDDM, HI 70/30						
Mean	**~8.2**	**7.70**	**11.12**	**3.42**	**14.12**	**8.58**
R=		0.39	0.52	0.25	0.56	0.50
N=		*44*	*42*	*42*	*42*	*36*
P=		0.01	N.S.	0.003	N.S.	N.S.
NIDDM, X-14 70/30						
Mean	**~7.9**	**8.48**	**10.08**	**1.60**	**13.93**	**8.53**
R=		0.25	0.35	0.21	0.36	0.40
N=		*76*	*75*	*75*	*75*	*69*
NIDDM, HI 70/30						
Mean	**~8.0**	**8.39**	**11.03**	**2.65**	**14.56**	**9.16**
R=		0.18	0.21	0.07	0.21	0.42
N=		*92*	*91*	*91*	*92*	*86*
P=		N.S.	0.12	0.05	N.S.	0.069

Patient values were included if they had an endpoint value for the particular glucose parameter and HgbA1c level. HgbA1c values varied slightly because the variances in the sample size for the glucose parameter—particularly the mean glucose profile.

R=Correlation coefficient of glucose parameter with HgbA1c

Fasting=Fasting glucose

PP=Post-prandial

Glucose excursion=90 minute post-breakfast glucose minus the fasting glucose *(Typically the most distinct excursions can be found in the morning because there is less carry-over from the prior insulin dose, e.g. Vol., p286.)*

AUC=area-under-the-curve estimate of glucose exposure at breakfast and for the subsequent 90 minutes

Mean=Mean glucose determined from glucometer logs with glucose measured at 8 time-points

Table 23

Relationship Between the Change in HgbA1c and the Mean Glucose at Endpoint*

	Delta HgbA1c (Last Visit-Baseline)(%)	Mean Glucose (mmol/l)	Correlation Coefficient	N=
IDDM X-14 70/30	-0.05	8.83	0.14	37
IDDM Human Insulin 70/30	-0.21	8.68	0.14	35
NIDDM X-14 70/30	-0.21	8.54	0.02	68
NIDDM Human Insulin 70/30	-0.15	9.16	0.22	86

*Eight point glucose profile as self-measured by patients using glucometers

Table 24

Relationship Between the Change in HgbA1c and the Glucose Excursion (90 Minute Post Breakfast Glucose Minus Fasting Glucose) at Endpoint*

	Delta HgbA1c (Last Visit-Baseline)(%)	Glucose Excursion (mmol/l)	Correlation Coefficient	N=
IDDM X-14 70/30	-0.007	0.47	-0.09	45
IDDM Human Insulin 70/30	-0.27	3.37	-0.03	41
NIDDM X-14 70/30	-0.21	1.62	-0.03	74
NIDDM Human Insulin 70/30	-0.12	2.65	0.17	91

*Glucose excursion as self-measured by patients using glucometers

13.--Safety Results

13.1.—General

The controlled studies were not sufficiently powered to identify adverse events other than those previously identified: hypoglycemia, changes in cross-reacting antibodies, and changes in alkaline phosphatase levels. The nature and number of adverse events as well as the number of withdrawals due to adverse events appeared to be comparable for the two treatment groups (Tables 25-26).

The extension studies were intended to provide long-term safety results—with the emphasis directed at the effect of antibodies on a) systemic-local allergic reactions and b) glycemic control and insulin doses. The raw data and results of these extension studies were not available for review.

Table 25
Drop-outs Due to Adverse Events

Tx	Pt #	Age	Gender	Event	Duration of Tx at Onset (Days)	Duration of Event (Days)
X-14	82	71	F	Diarrhea	2	10
X-14	170	68	M	Arterial thrombosis	83	6
X1-4	883	64	M	Rash, parasthesia	17/33	45/29
X-14	761	?	?	Unspecified	63	?
HI	389	47	F	Abdominal & back pain, nausea	6	9/16/38
HI	716	74	F	Neuropathy	4	81
HI	778	69	F	Rash-erythema	14	31

Tx=treatment Pt=patient F=female M=male HI=human insulin

Table 26
Serious Adverse Events

Tx	Pt #	Age	Gender	Event	Duration of Tx at Onset	Resulted in Withdrawal
X-14	81	47	M	Peripheral ischemia	49	No
X-14	170	68	M	Arterial thrombosis	83	YES
X1-4	411	55	M	Viral infection	8	No
X-14	818	63	M	Skin ulceration	3	No
HI	64	75	M	Hypoglycemia	7	No
HI	64	75	M	Hypoglycemia	12	No
HI	146	53	M	Urinary tract infection	51	No
HI	518	69	F	Bundle branch block	77	No
HI	567	67	F	Cranial nerve lesion	59	No
HI	572	75	F	Pancreatic carcinoma	-92	No
HI	716	74	F	Neuropathy	4	YES
HI	765	61	F	Uterine cancer	61	No
HI	864	66	F	Angina	52	No

Tx=treatment Pt=patient F=female M=male HI=human insulin

13.2.--Hypoglycemia

For the purposes of this review, hypoglycemia was defined as requiring intervention from a third party and/or having a blood glucose $\leq$36 mg/dl (2 mmol/L). This definition is relatively specific for clinically significant events and minimizes problems due to the relative inaccuracy of the home glucose meters and open-label nature of the trial. (See the minutes of the and the 1996 Winter and 1998 Spring E & M Advisory Committee meetings.)

Hypoglycemia, as is typical, was more common in the Type 1 patients (Table 27). Regardless of treatment arm, the median number of hypoglycemic events requiring third party intervention was zero for patients with IDDM. Hypoglycemia requiring third party intervention was limited to 15--20% of the exposed populations for both treatment arms. The overall rates of hypoglycemia requiring third party intervention, however, were approximately four times greater than the rates predicted by the DCCT for intensively

managed IDDM patients with HgbA1c values of ~8-8.5%, 0.40-0.45 events per patient-year regardless of treatment arm.

Table 27

Glycemic Control versus Hypoglycemia

(Hypoglycemia=glucose ≤36 mg/dl and/or requiring intervention from a third party)

Study 038 Treatment Group	HgbA1c (%)		Hypoglycemia--# Events							
			During Treatment Arm				During Final Month			
	End	Delta	Total	Blood Glucose </=2 mmol/L	Events Not Self Treated W/o Rx*	IV Glucose or Glucagon	Total	Blood Glucose </=2 mmol/L	Events Not Self Treated W/o Rx*	IV Glucose or Glucagon
X-14—IDDM Exposed=			32	18	8	6	6	0	2	4
N=			16	11	7	4	4	0	1	3
HI—IDDM Exposed=			42	18	18	6	11	4	4	3
N=			17	12	7	5	6	3	3	3
X-14—IDDM Exposed=			11	5	6	0	4	1	3	0
N=			7	5	3	0	2	1	1	0
HI—NIDDM Exposed=			20	8	10	2	5	3	2	0
N=			11	5	6	1	4	2	2	0

HI=human regular insulin compounds *W/o =without

X-14=insulin aspart compounds *Rx=IV glucose or glucagon

Hypoglycemia was less frequent in Type 2 patients, who typically have lower rates of hypoglycemia (Table 27). The median number of median number of hypoglycemic events requiring third party intervention was zero for patients with NIDDM. Hypoglycemia requiring third party intervention was limited to 3--8% of the exposed populations. There were only 2 events that required treatment with glucagon or IV glucose. These were found in the human insulin 70/30 group which had ~25% more patients than the X-14 70/30 treatment arm. The overall rates of hypoglycemia requiring third party intervention were similar to the rates predicted by the DCCT for intensively managed IDDM patients with HgbA1c values of ~8-8.5%.

Lastly, the occurrence of hypoglycemic events by time of day was similar regardless of treatment arm. The adjusted ANOVA of 2 A.M. glucometer readings did not show any difference by treatment group: 8.12 mmol/L (Vol.1, p296).

13.3.--Acidosis/Severe Hyperglycemia

In study 038, there were no cases of hyperglycemia or acidosis requiring hospitalization; It is unclear as to whether systematic monitoring was done for less serious cases of hyperglycemia or ketosis because neither the protocol nor the submission elaborates on this issue. Urine ketones were assessed at each visit at 2, 4, 8, and 12 weeks. Given the sporadic nature of ketosis, few events would be uncovered this way. Furthermore, it

15.5 and 20.5, females 17.6 and 31.8 nM) and AUC values, with no gender differences in plasma concentrations

Toxicology of aspart 30:
In acute toxicity studies in rats, single subcutaneous doses of aspart 30 up to 2000 U/kg (fresh and 3 months aged) were not lethal in rats, as no mortalities were noted. Clinical signs in rats at the above doses (2000 U/kg) were slight ptosis, piloerection, and decreased motor activity.

Local toxicity in pigs 2, 5 and 21 days after subcutaneous injection with aspart 30 formulation included mixed inflammatory cell infiltration, and clusters of crystals at 1-5 injection sites. These findings were not significantly different from the local changes produced by human insulin 30, or protaphane HM in pigs.

Fresh and aged aspart 30 produced significantly higher immune responses (7/10 and 9/10 rabbits had antibody responses) than either fresh and aged human insulin 30 (only 3/10 rabbits with fresh or aged drug had antibody responses). No significant immune response differences between aged and new biphasic aspart 30 or BHI 30 were observed. Both human insulin 30 and porcine insulin gave relatively low standard immune responses while bovine insulin control and aspart 30 gave relatively high standard immune responses. The sponsor explains that the high frequency of immune responses in the aspart 30 and bovine insulin were observed are because the amino acid (aa) compositions are two changes for aspart 30 and three changes for bovine insulin compared to rabbit insulin. Aspart 30 is only one amino acid different from human insulin.

Safety Evaluation: The primary issue of this formulation is the time-action profile of activity. The toxicity of insulin aspart has been well characterized in the approved NDA 20-986, and is in general similar to human insulin. Assuming the new mix is stable in a patient setting, there should be no unexpected toxicity with the proposed mixture

Conclusions:
The preclinical studies indicate that the proposed mixture appears to have faster onset of action and prolonged activity. From the limited pre-clinical toxicity data provided, no unexpected toxicity is expected with this mixture. The immunogenicity of the product in rabbits was higher than with human insulin 30, but sponsor explains that aspart differs from rabbit insulin by two amino acids, in contrast it differs from human insulin by only one amino acid. The approvability of the product will be determined from the pivotal human trial to determine its action and antigenicity in humans. From a pharmacology standpoint this NDA ——— is approvable.

Communication Review:

Labeling Review: Preclinical sections of the Label should be modified as proposed in the initial submission of NDA 20-986, which was approved in June 2000. The label for aspart 70/30 should read as follows:

Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of 70/30. In 52 week studies, **Sprauge-Dawley** rats **were** ~~with rats~~ dosed subcutaneously with **at 10, 50, and** 200 U/kg/day (approximately **2, 8, and** 32 times the human subcutaneous dose of 1.0 U/kg/day, based on body surface area).

was not genotoxic in the following tests, Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals

Pregnancy: Teratogenic effects: Pregnancy category **C**:

teratology studies have been performed with and regular human insulin in rats **and rabbits. In these studies, was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis.**

. The effects of did not differ from those observed with subcutaneous regular human insulin **like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32-times the human subcutaneous dose of 1.0 U/kg/day based on U/body surface area), and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/Kg/day for rats and equal to the**

[redacted] human subcutaneous dose of 1.0 U/kg/day for rabbits, based on U/body surface area.

[redacted] should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus

Nursing mothers- It is unknown whether [redacted] 70/30

[redacted]

Internal comments: AP

Recommendation:
From the preclinical standpoint, approval of this application is recommended, pending acceptable labeling modifications.

S

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cc: NDA Arch
HFD510
HFD510/antonipillai/elhage/koller/jrhee
Review Code: AP
Filename:nda21172

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Appendix 1: Table 1. Pharmacokinetic parameters of aspart 30 after single dose SC administration in rats.

	Low dose		High dose	
	Females 4.3 ± 0.2 U/kg	Males 3.1 ± 0.1 U/kg	Females 8.3 ± 0.4 U/kg	Males 6.1 ± 0.2 U/kg
AUC (pM * min)	67200	450172	1516118	873203
C_{max} (pM)	17539	15526	31809	20485
λ_z (min^{-1})	0.0072	0.0097	0.0066	0.0074
MRT (min)	47	36	68	69
$t_{1/2}$ (min)	96	72	105	94
t_{max} (min)	15	15	15	15

C_{max} ranged from 15526 to 31809 pM, indicating that the maximal plasma concentrations obtained by administering BIAsp 30 to Sprague Dawley Rats are sufficiently covered by the 52 week toxicity study in rats where plasma concentrations at 1 hour following administration were ranging [redacted] pM for male and [redacted] pM for female Sprague Dawley Rats.[11]

The pharmacokinetics of insulin aspart suggest the presence of linear pharmacokinetics when administering BIAsp 30 subcutaneously to Sprague Dawley Rats.

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Table 2. Pharmacokinetic parameters of aspart 30 at pH 7.2 and pH 7.4 vs that of human insulin 30 in pigs

Mean (SD)	*BIAsp30 pH=7.2*	*BIAsp30 pH=7.4*	*BHI 30*
C_{max} (pM)	349 (328)	400 (252)	361 (163)
t_{max} (min)	52 (33)	66 (76)	231 (245)
AUC (pM * min)	71518 (35224)	59509 (25280)	230714 (73036)
MRT (min)	426 (138)	453 (228)	882 (260)
λ_z (min^{-1})	0.0075 (0.0033)	0.0085 (0.0055)	0.0040 (0.0020)
$t_{½}$ (min)(harmonic mean)	93 ———	82 ———	174 ———

Comparison of the pharmacokinetic parameters between the three preparations were carried out by statistical analysis which is presented below.

Table 3. Pharmacokinetic parameters of five preparations of aspart 30 in different ratios

Table 10 Pharmacokinetic parameters mean (SD or range) for the five ratios of BIAsp following SC administration of 0.15 U/kg to eight Pigs (P-7)

Mean	*BIAsp100:0*	*BIAsp 30:70*	*BIAsp 50:50*	*BIAsp70:30*
C_{max} (pM)	731	181	242	454
(SD)	(392)	(80)	(83)	(343)
t_{max} (min)	45**	35**	35**	25**
(range)		[redacted]		
AUC (pM*min)	49287	23086	26810	27534
(SD)	(35184)	(11906)	(8244)	(16656)
MRT (min)	92	180	202	119
(SD)	(13)	(75)	(73)	(70)
λ_z (min^{-1})	0.0159	0.0082	0.0062	0.0107
(SD)	(0.0048)	(0.0046)	(0.0037)	(0.0044)
$t_{½}$ (min)	44*	84*	113*	65*
(range)		[redacted]		

* Harmonic mean
** Median

Table 4. Pharmacodynamic parameters of aspart 30 vs human insulin 30 in pigs

Table 2 Pharmacodynamic parameters (P-2)

Mean (SEM)	*BIAsp30*	*BHI 30*	*p-value (paired/unpaired t-test)*
Slope, (mM min)	-0.033 (0.004)	-0.021 (0.004)	0.12/<0.05
t_{min}(min)	129 (23)	176 (20)	0.24/0.15*
Δ Blood glucose(mM)	-1.05 (0.25)	-0.20 (0.26)	0.13/<0.05

*statistical test of limited relevance

This study indicated that compared to BHI 30, BIAsp 30 had a faster onset of action, as it reached the maximum effect earlier and exerted a more pronounced effect at the peak level.

Figure 1. Glucose lowering curves for BIASP 30, 50, 70 and 100 (IASP)