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ORIGINAL ARTICLE

Single-dose euglycaemic clamp studies demonstrating pharmacokinetic and pharmacodynamic similarity between MK-1293 insulin glargine and originator insulin glargine (Lantus) in subjects with type 1 diabetes and healthy subjects

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Aims: MK-1293 is an insulin glargine that has an amino acid sequence identical to that of Lantus, the originator insulin glargine. Two euglycaemic clamp studies, 1 in subjects with type 1 diabetes (T1D) and 1 in healthy subjects, were conducted to demonstrate pharmacokinetic (PK) and pharmacodynamic (PD) similarity between MK-1293 and Lantus commercially procured in both the European Union (EU-Lantus) and the USA (US-Lantus).

Materials and Methods: Both studies were single-dose, randomized, double-blind, single-centre, crossover studies with ≥7 days between dosing periods. A 2-treatment, 4-period replicate crossover study in T1D subjects (N = 76) compared the PK and PD of MK-1293 to EU-Lantus for 30 hours after dosing. A 3-period crossover study in healthy subjects (N = 109) compared the PK and PD of MK-1293, EU-Lantus and US-Lantus for 24 hours after dosing. In both studies, all subjects received single 0.4 units/kg subcutaneous doses of MK-1293 or Lantus in all dosing periods. Pharmacokinetic assessment was based on LC-MS/MS-based measurement of the major insulin glargine metabolite (M1) and PD was characterized using the euglycaemic clamp platform.

Results: In both studies, pre-specified similarity criteria were met between MK-1293 and Lantus for comparison of PK ($AUC_{0\text{-}24}$ and C_{max} of M1) and PD (GIR-$AUC_{0\text{-}24}$, GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$, and GIR_{max}) primary endpoints. All treatments were well tolerated.

Conclusion: Based on comparative assessment in both T1D and healthy subjects, it can be concluded that the PK and PD properties of MK-1293 are highly similar to those of Lantus. (ClinicalTrials.gov: NCT02059174).

KEYWORDS

biosimilar insulin, glycaemic control, insulin analogues, pharmacodynamics, pharmacokinetics, type 1 diabetes

1 | INTRODUCTION

Insulin glargine is a recombinant human basal insulin analog produced in *Escherichia coli* (*E. coli*) that is marketed worldwide by Sanofi (Paris, France) under the trade name Lantus. Lantus is approved for the treatment of type 1 diabetes (T1D) and type 2 diabetes (T2D).[1,2] Though Lantus and other approved basal insulin products are effective treatments for patients with T1D and T2D, the cost of these products can present a barrier to patient access. High-quality follow-on basal insulin products, approved for marketing based on rigourous

demonstration of similarity to approved reference products, are important treatment options that could increase access for a broader population of patients. The specific terminology applied to follow-on insulin products such as MK-1293 differs among regulatory regions. They are designated as biosimilars in several regions including the European Union.[3] In the USA, while follow-on insulin products are not currently designated as biosimilars and are developed under a different regulatory pathway,[4] the same principle of establishing similarity to an approved reference product is applied.

Demonstration of similarity between follow-on and approved reference insulin products is based on a combination of analytical, preclinical and clinical data. Clinical similarity is assessed through Phase I comparisons of pharmacokinetic (PK) and pharmacodynamic (PD) properties as well as efficacy and safety comparisons in the Phase III setting. Because of the high sensitivity of PK and PD comparisons for discriminating potentially relevant differences between insulins, Phase I euglycaemic clamp studies play a central role in the assessment of clinical similarity.[3]

MK-1293 is a follow-on insulin glargine that has the same amino acid sequence as Lantus and, like Lantus, is produced in *E. coli*. Similarity with regard to clinical efficacy and safety have been demonstrated between MK-1293 and Lantus in Phase III studies of subjects with both T1D[5] and T2D.[6] These studies demonstrated equivalent HbA1c reductions from baseline between MK-1293 and Lantus at highly similar doses, as well as similarity with regard to hypoglycaemia, body weight, general safety and immunogenicity.

The present paper reports on 2 euglycaemic clamp studies conducted to compare the clinical PK and PD properties of MK-1293 and Lantus. Because of differing cross-regional regulatory requirements, Study A compared MK-1293 and Lantus commercially procured in the EU (EU-Lantus) in T1D subjects, while Study B was conducted in healthy subjects and compared MK-1293, EU-Lantus and Lantus commercially procured in the USA (US-Lantus).

2 | RESEARCH DESIGN AND METHODS

2.1 | Study design

Study A (NCT02059174; Protocol Number [PN] 005) was a double-blind, randomized, single-centre, 2-treatment, 4-period replicate crossover study in T1D subjects (Figure S1A). In each period, eligible subjects received 1of 2 single-dose treatments (MK-1293 or EU-Lantus) in 1 of 2 treatment sequences (A-B-A-B or the reverse). All subjects completing the study received both treatments twice. Subjects receiving insulin glargine at screening were transitioned to insulin detemir for the duration of the study. Within 36 hours of dosing, the only basal insulin permitted was NPH, which could be administered up to 22 hours prior to dosing. After the evening snack and approximately 10 hours prior to dosing, an overnight intravenous infusion of insulin aspart (Novolog) was initiated to ensure stabilization of plasma glucose concentration at approximately the clamp target (130 mg/dL [7.22 mmol/L]) by the time of dosing.

Study B (PN002) was a double-blind, randomized, single-centre, 3-period complete crossover study in healthy subjects (Figure S1B). In each period, eligible subjects received 1 of 3 single-dose treatments (MK-1293, US-Lantus or EU-Lantus) in randomized order. All subjects completing the study received each treatment once. The plasma glucose concentration clamp target in Study B was 80 mg/dL (4.4 mmol/L).

In both studies, subjects were admitted to the clinical research unit (CRU) 24 to 36 hours prior to dosing and dosing occurred on the morning of Day 1 after an overnight fast of at least 10 hours. All doses of MK-1293 and Lantus were 0.4 units/kg, administered by subcutaneous (s.c.) injection in the abdomen. Dosing was followed in each period by 30 (Study A) or 24 (Study B) hours of PK sampling and euglycaemic clamping. Subjects remained on the CRU for approximately 48 (Study A) or 30 (Study B) hours post dose. There was a minimum of 7 days between dosing in each treatment period.

Both studies were conducted in accordance with the guidelines on good clinical practice and with the ethical standards for human experimentation established by the Declaration of Helsinki and were approved by the appropriate institutional review boards and regulatory agencies. All patients provided written informed consent prior to participating in the trials.

2.2 | Study drugs

MK-1293 was manufactured by Merck & Co., Inc., Kenilworth, New Jersey and was provided in cartridges containing MK-1293 at a concentration of 100 units/mL. EU-Lantus was procured commercially from the EU market as cartridges compatible with commercial reusable pen systems (ie, Tactipen, Sanofi, Paris, France). US-Lantus was procured from the US market as Solostar Disposable Pens (Sanofi, Paris, France). To maintain double-blinding and consistency of dosing methodology across treatments, MK-1293, US-Lantus and EU-Lantus were withdrawn by an unblinded pharmacist from cartridges/pens with insulin syringes using a process consistent with Lantus product labeling.[1]

2.3 | Study subjects

In Study A, subjects were men and women aged 18 to 65 years with a clinical diagnosis of T1D for ≥12 months, C-peptide concentration ≤0.7 ng/mL (≤0.23 nmol/L) with a concurrent plasma glucose concentration >90 mg/dL (5 mmol/L), HbA1c of <9.5%, BMI 18.0 to 30.0 kg/m^2 and an estimated (Modification of Diet in Renal Disease equation) glomerular filtration rate of ≥60 mL/min/m^2. Total daily insulin dose (basal plus prandial) must have been ≤1.2 units/kg per day and stable (±20%) for 2 weeks prior to screening. Subjects with hyperlipidaemia and/or hypertension being treated with lifestyle modification or a stable medical regimen were permitted, as were subjects with non-proliferative diabetic retinopathy, microalbuminurea and peripheral diabetic neuropathy. Subjects with severe hypoglycaemic episodes associated with seizure or coma within 3 months of screening or diabetic ketoacidosis within 6 months of screening were excluded.

In Study B, subjects were healthy men aged 18 to 45 years, with BMI of 18.5 to 25.0 kg/m^2, fasting plasma glucose (FPG) >75 and <100 mg/dL (>4.1 and <5.5 mmol/L) and creatinine clearance

>80 mL/min based on the Cockroft-Gault equation or 24-hour urine collection.

2.4 | Pharmacokinetic sampling

Plasma samples were collected for PK analysis in Study A at −3 minutes prior to dosing and 1, 4, 8, 10, 12, 16, 20, 24, 27 and 30 hours post dose and in Study B at −3 minutes prior to dosing and 1, 4, 8, 10, 12, 14, 16, 18, 20 and 24 hours post-dose.

2.5 | Euglycaemic clamping

In both studies, subjects began fasting from 10:00 PM on Day −1 (day prior to dosing) in each period and remained fasting for 30 (Study A) or 24 (Study B) hours after dosing. A dorsal hand vein or lateral wrist vein was cannulated prior to dosing for frequent blood glucose measurements using an automatic blood glucose analyzer (GlucoScout I-01G, International Biomedical, Austin, Texas), with measurements taken at least every 5 minutes starting at 0 hours post dose and continuing through the completion of clamping. GlucoScout measurements were verified at regular intervals (approximately every 30 minutes) with a YSI 2300 STAT (YSI Life Sciences, Yellow Springs, Ohio) Glucose Analyzer. The antecubital vein of the same arm was cannulated for serial measurements of serum insulin and blood glucose concentration. The contralateral arm or hand vein was cannulated for the infusion of glucose (20% dextrose solution in water).

In Study A, the clamp target was 130 mg/dL which was maintained initially through weaning of the overnight insulin infusion and then through initiation and adjustment of a glucose infusion. In Study B, the clamp target was 80 mg/dL, which was maintained through glucose infusion rate (GIR) adjustment, as needed. For both studies, a recommended GIR was calculated at each blood glucose sampling time point by a model-based algorithm and refined by the clamp director if necessary. The glucose infusion was discontinued by 30 (Study A) or 24 (Study B) hours after dosing, or earlier if not needed to maintain the clamp target blood glucose concentration.

2.6 | Bioanalytical methods

In both studies, a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) detection method, modified from a previously published assay,[7] was used to simultaneously determine concentrations of parent insulin glargine and its 2 bioactive metabolites, M1 and M2. The method was based on an automated 96-well format immunoaffinity purification of analytes of interest from human plasma. Parent glargine, M1, M2 and their corresponding stable isotope-labeled internal standards were chromatographed using reversed phase LC and were detected with tandem mass spectrometric detection using multiple reaction montoring (MRM). The analytical range of quantification was 100 to 10 000 pg/mL for all analytes using 0.5 mL of plasma. All validation and sample quantification runs met the pre-specified acceptance criteria, including incurred sample reproducibility (ISR).

2.7 | Pharmacokinetic assessments

As M1 is the major circulating active glargine species after s.c. administration,[7–9] it was the primary PK analyte. Pharmacokinetic parameters included $AUC_{0\text{-}24}$ and C_{max} (primary endpoints), as well as $AUC_{0\text{-}12}$, $AUC_{12\text{-}24}$ and T_{max}. The values of all individual PK parameters were calculated using noncompartmental methods in Phoenix WinNonlin v6.3 (Certara, St. Louis, Missouri). Areas under the curves (AUCs) were calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations (linear-up/log-down). A uniform weighting scheme was used. C_{max} and T_{max} were assessed directly from the observed concentration-time data taken directly from the bioanalytical data. Consistent with regulatory guidance,[10] values of M1 below the lower limit of quantitation (LLOQ) were imputed as zero. Plasma concentrations for parent glargine and the M2 glargine metabolite were below the LLOQ for the majority of samples at all PK sampling time points. Therefore, no PK parameters were calculated for these analytes.

2.8 | Pharmacodynamic assessments

GIR parameters in both studies included area under the GIR profile from 0 to 24 hours (GIR-$AUC_{0\text{-}24}$), GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$ and maximum GIR value (GIR_{max}) (primary endpoints), as well as time to GIR_{max}. GIR_{max} is based on LOESS smoothed data where the AUC endpoints are based on observed data. The default smoothing function in SAS PROC LOESS, which selects an optimal smoothing parameter based on minimizing a bias-corrected AIC criterion, was used to fit an optimal smoothing function for each subject, treatment and period. In Study A, Duration of Action (DOA) was also a primary PD endpoint, defined as the length of time from dosing to end of action, the time point at which plasma glucose was >150 mg/dL for 30 minutes with no glucose infusion during that time.[11] There were 3 instances (of 290 total clamps) in which application of the end of action definition above clearly under-estimated the timing of end of action and the pre-specified definition of end of action was overruled. As end of action does not clearly manifest in healthy subjects, DOA was not assessed in Study B.

2.9 | Safety assessments

Adverse events (AEs) were assessed throughout the 2 studies. All clinical AEs were evaluated in terms of intensity, duration, severity, outcome and relationship to study medication. Other safety parameters, including vital signs, physical examinations, 12-lead electrocardiograms (ECGs), and standard laboratory safety tests, were assessed pre-dose and at various time points post-dose and post-study.

2.10 | Statistical analysis

2.10.1 | Pharmacokinetics

Individual M1 metabolite $AUC_{0\text{-}24}$ and C_{max} values were natural log-transformed and analysed separately in linear mixed effects models appropriate for either a 2-treatment 4-period replicate crossover (Study A) or a 3-treatment 3-period crossover (Study B). A 90% CI for the difference in treatment means for pairwise treatment

comparisons on the log scale was calculated using the mean square error from the model and referencing a t-distribution for each endpoint. The CIs were exponentiated to obtain the 90% CI for the geometric mean ratio for the treatment comparisons for $AUC_{0\text{-}24}$ and C_{max}. Consistent with pertinent regulatory agency guidelines,[3,12–14] a conclusion of PK similarity for each study and treatment comparison would be supported if the 90% CIs for both $AUC_{0\text{-}24}$ and C_{max} for a given treatment comparison were within the predefined similarity criteria of 0.80 and 1.25. The secondary endpoints of M1 $AUC_{0\text{-}12}$ and $AUC_{12\text{-}24}$ were analysed using the same statistical methods as described for M1 $AUC_{0\text{-}24}$ and C_{max}.

2.10.2 | Pharmacodynamics

To assess glucose concentration variability around the clamp target, a mixed effects model with only a random effect for subject was used to estimate the within-subject glucose variability across time points and periods. Mean ambient glucose concentration and within-subject percent coefficient of variation (% CV) were calculated from the model using glucose concentrations between the initiation of glucose infusion and either the last time glucose was infused during the clamp (Study B) or the last time glucose was infused prior to end of action (Study A).

Individual GIR parameter (GIR-$AUC_{0\text{-}24}$, GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$ and GIR_{max}) values were analysed with a linear mixed effects model with fixed effects terms for treatment and period. Fieller's Theorem[15] was used to calculate 95% (Study A) or 90% (Study B) CIs for the ratio of arithmetic means (test/reference) for each parameter and treatment comparison, using results from the linear mixed effects model and referencing a t-distribution. The use of 90% compared with 95% CIs was because of differing cross-regional regulatory agency requirements. In Study A, a conclusion of PD similarity between MK-1293 and EU-Lantus would be supported if the 95% CIs for the ratio of arithmetic means for all 4 primary GIR endpoints, as well as DOA, lay within the pre-specified boundaries. However, end of action did not occur within the 30-hour clamp timeframe in the majority of subjects for both treatments. Therefore, a DOA comparison based on a ratio of means could not be performed and a statistical comparison of DOA was conducted with a *post-hoc* survival analysis approach using hazard rates. A frailty model was used with effects for treatment, period and sequence, and a random effect for subject. From this model, the hazard ratio and Wald 95% CIs for treatment were obtained. A value of 1.00 for the hazard ratio corresponds to no difference between treatments. In Study B, a conclusion of PD similarity for each treatment comparison would be supported if the 90% CIs for the ratio of arithmetic means for all 4 primary GIR endpoints lay within (0.80, 1.25).

2.10.3 | Sample size

For both studies, an overall power of 80% to meet all study objectives was targeted. In the Study A protocol, assuming a 2-sided alpha of 0.025 for PD (95% CIs) and 0.05 for PK (90% CIs), and similarity bounds for the primary PK and PD endpoints as noted above, a sample size of 70 completers was predicted to provide at least 80% overall power. In the Study B protocol, assuming a 2-sided alpha of 0.05 (90% CIs) for all primary PK and PD endpoints and the similarity bounds noted above, a sample size of 72 completers was predicted to provide at least 80% overall power. Following the start of each study, an interim analysis for assessment of variance for the primary PK and PD endpoints was conducted to determine whether it was necessary to increase sample size to provide at least 80% overall power to meet all hypotheses. Mean treatment effects were not calculated during the interim analyses. These interim variance assessments were conducted by a pharmacokineticist and a statistician who were blinded to treatment and had no other involvement in these studies. For Study A, no increase in sample size was needed. For Study B, the interim analysis indicated that a sample size of 72 would not have provided at least 80% overall power; therefore, the sample size was increased from 72 to 96 completers.

3 | RESULTS

3.1 | T1D (Study A)

3.1.1 | Demographics and disposition

A total of 76 subjects with T1D (mean age, 33.4 years) participated in Study A, of whom 70 completed the study. Subject demographics and baseline characteristics for Study A are presented in Table 1. Of the 6 subjects who did not complete the study, 4 were withdrawn because of subject decision, 1 because of glucose infusion error, and 1 because of physician decision as the patient required antibiotic treatment.

3.1.2 | Pharmacokinetics

Mean PK profiles for the M1 glargine metabolite were similar between MK-1293 and EU-Lantus in subjects with T1D (Figure 1A). The 90% CIs for the geometric mean ratios (MK-1293/Lantus) were within 0.80 and 1.25 for both primary PK endpoints ($AUC_{0\text{-}24}$ and C_{max}), thereby meeting the pre-specified statistical similarity criterion (Table 2). For both secondary PK endpoints of interest ($AUC_{0\text{-}12}$ and $AUC_{12\text{-}24}$), the 90% CIs for the geometric mean ratios (MK-1293/Lantus) were also within 0.80 and 1.25, although no statistical similarity criteria were pre-specified for these comparisons. Median M1 T_{max} was approximately 12 hours for both treatments. The replicate design of Study A

TABLE 1 Demographics and baseline characteristics

	T1D (Study A) N = 76	Healthy volunteers (Study B) N = 109
Age, years	33.4 ± 11.7	28.1 ± 6.5
Males	48 (63.2)	109 (100)
Body mass index, kg/m^2	23.3 ± 1.4	25.2 ± 2.4
Race		
American Indian or Alaska Native	1 (1.3)	1 (0.9)
Asian	2 (2.6)	5 (4.6)
Black or African American	2 (2.6)	21 (19.3)
Multiple	0	2 (1.8)
Native Hawaiian or Other Pacific Islander	1 (1.3)	1 (0.9)
White	70 (92.1)	79 (72.5)

Data are expressed as mean ± SD or n (%).

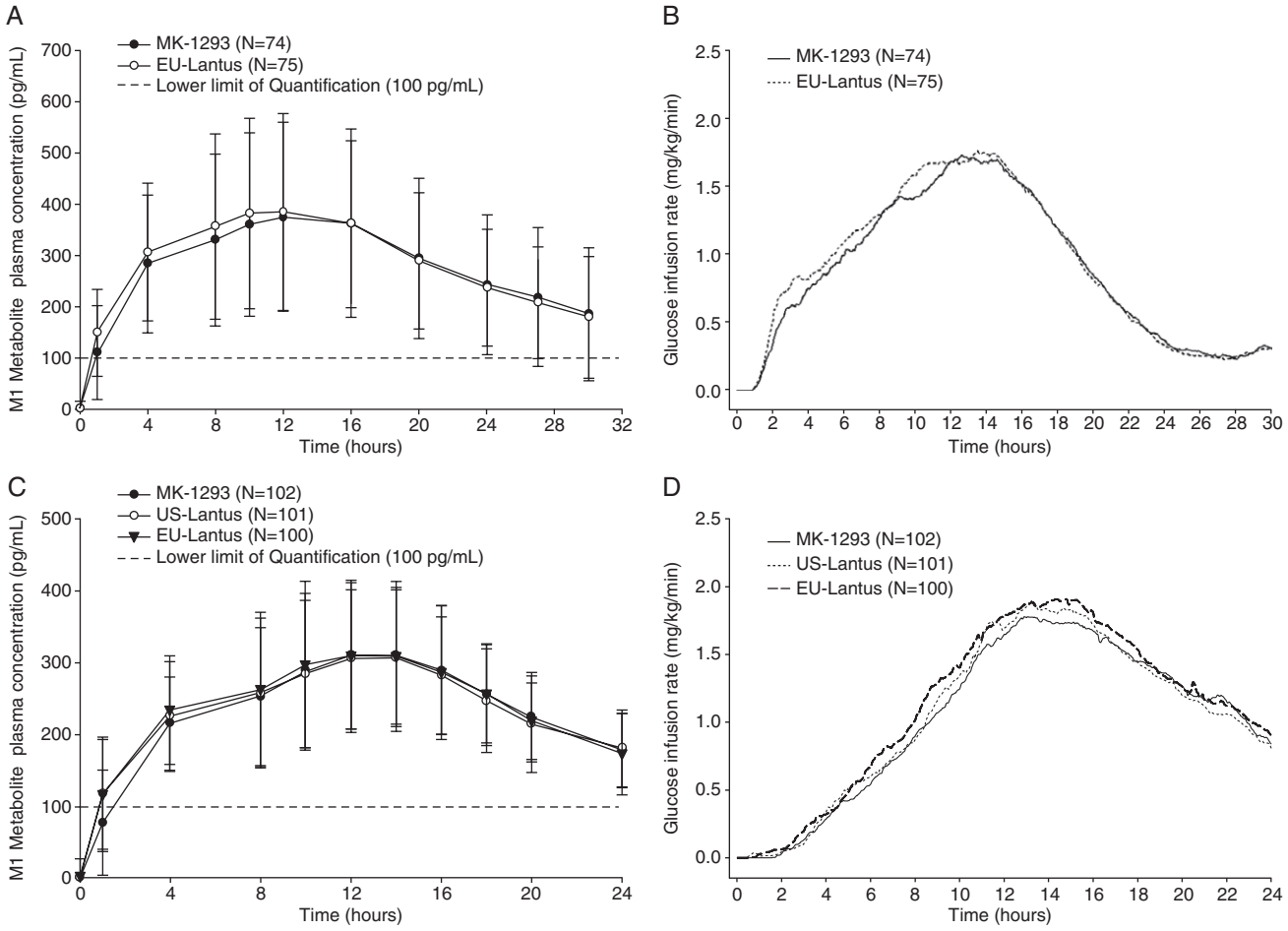

FIGURE 1 A, Plot of mean ± SD M1 glargine metabolite plasma concentrations over time during a 30-hour euglycaemic clamp following single 0.4 units/kg s.c. doses of MK-1293 and EU-Lantus in subjects with type 1 diabetes. B, Plot of mean glucose infusion rates (GIRs) during a 30-hour euglycaemic clamp following single 0.4 units/kg s.c. doses of EU-Lantus and MK-1293 in subjects with type 1 diabetes. C, Plot of mean ± SD M1 glargine metabolite plasma concentrations over time during a 24-hour euglycaemic clamp following single 0.4 units/kg s.c. doses of EU-Lantus, US-Lantus and MK-1293 in healthy subjects. D, Plot of mean GIRs during a 24-hour euglycaemic clamp following single 0.4 units/kg s.c. doses of EU-Lantus, US-Lantus and MK-1293 in healthy subjects

permitted assessment of within-subject, between-period PK variability. The within-subject % CV for MK-1293 was consistent with that of EU-Lantus for all primary and secondary PK endpoints.

3.1.3 | Pharmacodynamics

Between the time of dosing and end of action, mean plasma glucose concentration was very close to the clamp target of 130 mg/dL (129.5 mg/dL with a % CV of 5.9%). This is consistent with the reported clamping precision in T1D subjects[10,11] and confirms that glucose clamping was achieved with a high degree of precision. Mean GIR profiles were similar between MK-1293 and EU-Lantus (Figure 1B) and the 95% CIs for the ratios of arithmetic means (MK-1293/Lantus) for all 4 GIR-based primary PD endpoints (GIR-$AUC_{0\text{-}24}$, GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$, GIR_{max}) were within 0.80 and 1.25, thereby meeting the pre-specified statistical similarity criterion for these endpoints (Table 2). Median Time to GIR_{max} was approximately 10 to 11 hours across treatments. As with PK, the within-subject % CV for MK-1293 was consistent with that of EU-Lantus for all primary GIR endpoints. For both MK-1293 and Lantus, end of action was not observed during the 30-hour clamping timeframe in the majority of subjects. Both a statistical survival analysis (Table 2) and the distribution of DOA (Figure S2) indicated similarity between MK-1293 and EU-Lantus.

3.2 | Healthy subjects (Study B)

3.2.1 | Demographics and disposition

A total of 109 healthy volunteers (mean age, 28.1 years) participated in Study B, of whom 96 completed the study. Subject demographics and baseline characteristics for Study B are presented in Table 1. Of the 13 subjects who did not complete the study, 8 were withdrawn because of subject decision, 1 because of technical clamp failure, 1 because of elevated fasting glucose, and 3 because of physician decision following non-compliance or protocol violations.

3.2.2 | Pharmacokinetics

Mean PK profiles for the M1 glargine metabolite were similar among all 3 treatments in healthy volunteers (Figure 1C). The 90% CIs for the geometric mean ratios (test/reference) for all 3 treatment comparisons were within 0.80 and 1.25 for both primary PK endpoints

TABLE 2 Primary and secondary PK and PD endpoints in T1D (Study A)

Endpoint	MK-1293		EU-Lantus		Mean ratio MK-1293 / EU-Lantus[c] (90% CI PK and 95% CI PD)	Within-subject % CV	
	N[a]	Mean (95% CI)[b]	N[a]	Mean (95% CI)[b]		MK-1293	EU-Lantus
PK endpoints							
$AUC_{0\text{-}24}$ (pg·h/mL)[d]	74	6530 (5967, 7145)	75	6763 (6162, 7423)	0.97 (0.91, 1.02)	23.6	31.6
C_{max} (pg/mL)[d]	74	372 (339, 408)	75	382 (350, 416)	0.97 (0.93, 1.03)	26.4	27.8
$AUC_{0\text{-}12}$ (pg·h/mL)[d]	74	2981 (2714, 3274)	75	3251 (2968, 3562)	0.92 (0.86, 0.97)	28.4	32.7
$AUC_{12\text{-}24}$ (pg·h/mL)[d]	74	3510 (3205, 3845)	75	3522 (3228, 3844)	1.00 (0.95, 1.04)	21.7	24.5
T_{max} (h)[e]	74	12.5 (4.0, 21.5)	75	12.0 (4.0, 23.0)	-	-	-
PD endpoints							
GIR $AUC_{0\text{-}24}$ (mg/kg)	74	1470.07 (1256.52, 1683.63)	74	1554.53 (1334.03, 1775.04)	0.95 (0.88, 1.01)	30.1	37.1
GIR $AUC_{0\text{-}12}$ (mg/kg)[a]	74	659.38 (557.84, 760.92)	75	736.31 (625.22, 847.41)	0.90 (0.81, 0.99)	39.5	46.6
GIR $AUC_{12\text{-}24}$ (mg/kg)	74	811.43 (689.89, 932.98)	74	818.20 (700.40, 936.01)	0.99 (0.92, 1.06)	28.1	37.4
GIR_{max} (mg/kg/min)[f]	74	2.34 (2.11, 2.57)	74	2.43 (2.18, 2.68)	0.96 (0.91, 1.02)	27.9	33.5
Time to GIR_{max} (h)[e,f]	74	10.28 (2.85, 19.13)	74	11.09 (3.23, 16.77)	-	-	-
DOA hazard ratio	74	–	-	-	1.07 (0.72, 1.59)	-	-

Abbreviations: % CV, percent coefficient of variation; AUC, area under the curve; C_{max}, maximum plasma concentration; DOA, duration of action; GIR, glucose infusion rate; GIR_{max}, maximal glucose infusion rate; PD, pharmacodynamic; PK, pharmacokinetic .

[a] Number of subjects with at least 1 administration of the particular treatment. (For PD, 1 subject with valid clamp data only to 18.5 hours after administration of EU-Lantus in 1 period because of an error in clamping execution. Subsequently discontinued from study participation. Only GIR $AUC_{0\text{-}12}$ calculated from the single period of EU-Lantus administration.)

[b] PK mean endpoint results presented as geometric means with 95% CI's; PD mean endpoint results presented as arithmetic means with 95% CI's.

[c] PK endpoint mean ratio results presented as geometric mean ratios with 90% CI's; PD endpoint mean ratio results presented as arithmetic mean ratios with 95% CI's (95% CI's calculated via Fieller's Theorem); PD post-hoc survival analysis endpoint presented as DOA hazard ratio and 95% CI.

[d] Analysis performed on log scale; results back transformed to original scale.

[e] Median (min, max) (calculated after taking the median of time to T_{max} [for PK] or GIR_{max} [for PD] values across the replicates for given subject and treatment).

[f] Determined from smoothed data.

($AUC_{0\text{-}24}$ and C_{max}), thereby meeting the pre-specified statistical similarity criterion (Table 3). For both secondary PK endpoints of interest ($AUC_{0\text{-}12}$ and $AUC_{12\text{-}24}$), the 90% CIs for the geometric mean ratios (MK-1293/Lantus) were also within 0.80 and 1.25 for all 3 treatment comparisons, although no statistical similarity criteria were pre-specified for these comparisons. Median M1 T_{max} was 13 to 14 hours across treatments.

3.2.3 | Pharmacodynamics

During the timeframe of active glucose clamping (from initiation to completion of glucose infusion), mean plasma glucose concentration was very close to the clamp target of 80 mg/dL (80.6 mg/dL with a coefficient of variation of 4.7%). This is consistent with the reported clamping precision in similar studies and confirms that glucose clamping was achieved with a high degree of precision.[16,17] Mean GIR profiles were similar among all treatments (Figure 1D). The 90% CIs for the arithmetic mean ratios (test/reference) for all 3 treatment comparisons were within 0.80 and 1.25 for all 4 primary GIR endpoints (GIR-$AUC_{0\text{-}24}$, GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$, GIR_{max}), thereby meeting the pre-specified statistical similarity criterion (Table 3). Median Time to GIR_{max} was approximately 13 hours for all treatments.

3.3 | Safety: Studies A (T1D subjects) and B (healthy subjects)

MK-1293 was generally well tolerated in both studies. No serious adverse events, deaths or discontinuations for safety/tolerability reasons occurred in either study. In each study, similar proportions of subjects reported adverse events after treatment with MK-1293 and the Lantus comparator(s) and all adverse events were mild to moderate in intensity. In Study B (healthy subjects), 8 (7.8%) subjects reported injection-site pain after MK-1293 administration, compared to 5 (5%) and 2 (2%) subjects after US-Lantus and EU-Lantus, respectively. In Study A (T1D subjects), 2 (2.7%) and 1 (1.3%) subjects reported injection-site pain after MK-1293 and EU-Lantus, respectively. There were no consistent treatment-related changes in laboratory, vital sign or ECG safety parameter values in either study.

4 | DISCUSSION

MK-1293 is an insulin glargine being developed for the treatment of T1D and T2D. The principal goal of the 2 euglycaemic clamp studies reported here was to demonstrate PK and PD similarity between MK-1293 and Lantus. Though Lantus commercially available in the EU and USA has the same manufacturer and drug product composition,[1,2] cross-regional regulatory agency requirements necessitated direct demonstration of PK and PD similarity between MK-1293 and Lantus procured in both the EU and the USA, as well as confirmation of the PK and PD similarity of EU and US-Lantus. These goals were successfully achieved and the results of the studies supported the recent approval of MK-1293 as a biosimilar insulin glargine in the EU (January 2017).

TABLE 3 Primary and secondary PK and PD endpoints in healthy volunteers (Study B)

	MK-1293		US-Lantus		EU-Lantus		Mean Ratio (90% CI)[a]		
Endpoint	N[b]	Mean (95% CI)[c]	N[d]	Mean (95% CI)[c]	N	Mean (95% CI)[c]	MK-1293 / US-Lantus	MK-1293 / EU-Lantus	EU-Lantus / US-Lantus
PK endpoints									
$AUC_{0\text{-}24}$ (pg·h/mL)[e]	102	5335 (4994, 5699)	100	5455 (5169, 5757)	100	5526 (5219, 5850)	0.98 (0.93, 1.03)	0.97 (0.92, 1.02)	1.01 (0.97, 1.05)
C_{max} (pg/mL)[e]	102	323 (305, 343)	100	319 (301, 339)	100	323 (303, 344)	1.01 (0.96, 1.07)	1.00 (0.95, 1.05)	1.01 (0.96, 1.06)
$AUC_{0\text{-}12}$ (pg·h/mL)[e]	102	2415 (2256, 2584)	101	2539 (2380, 2709)	100	2583 (2412, 2765)	0.95 (0.90, 1.01)	0.93 (0.89, 0.98)	1.02 (0.97, 1.07)
$AUC_{12\text{-}24}$ (pg·h/mL)[e]	102	2927 (2765, 3100)	100	2880 (2739, 3028)	100	2909 (2760, 3065)	1.02 (0.97, 1.07)	1.01 (0.96, 1.05)	1.01 (0.97, 1.05)
T_{max} (h)[f]	102	14.0 (4.0, 20.0)	100	13.0 (4.0, 24.0)	100	14.0 (1.0, 24.0)	-	-	-
PD endpoints									
GIR $AUC_{0\text{-}24}$ (mg/kg)	102	1533.12 (1296.33, 1769.92)	100	1517.27 (1299.26, 1735.29)	100	1598.09 (1355.67, 1840.50)	1.01 (0.92, 1.10)	0.96 (0.89, 1.04)	1.05 (0.97, 1.14)
GIR $AUC_{0\text{-}12}$ (mg/kg)	102	475.87 (376.56, 575.18)	101	495.87 (400.16, 591.58)	100	522.58 (413.35, 631.82)	0.96 (0.84, 1.09)	0.91 (0.82, 1.02)	1.05 (0.93, 1.19)
GIR $AUC_{12\text{-}24}$ (mg/kg)	102	1055.07 (912.03, 1198.11)	100	1023.57 (891.55, 1155.59)	100	1075.60 (931.28, 1219.92)	1.03 (0.95, 1.12)	0.98 (0.91, 1.05)	1.05 (0.97, 1.14)
GIR_{max} (mg/kg/min)[g]	102	2.26 (1.94, 2.59)	100	2.36 (1.99, 2.73)	100	2.48 (2.10, 2.87)	0.96 (0.87, 1.06)	0.91 (0.84, 0.99)	1.05 (0.95, 1.17)
Time to GIR_{max} (h)[f,g]	102	13.1 (4.3, 23.9)	100	13.1 (0.0, 23.8)	100	13.2 (0.0, 23.0)	-	-	-
Onset of action (h)	102	2.10 (1.81)	101	1.96 (1.62)	100	1.89 (1.46)	-	-	-

Abbreviations: AUC, area under the curve; C_{max}, maximum plasma concentration; GIR, glucose infusion rate; GIR_{max}, maximal glucose infusion rate; PD, pharmacodynamic; PK, pharmacokinetic; SD, standard deviation.

[a] PK endpoint mean ratio results presented as geometric mean ratios with 90% CI's; PD endpoint mean ratio results presented as arithmetic mean ratios with 90% CI's (90% CI's calculated via Fieller's Theorem); onset of action presented as non-model-based mean (SD).

[b] One subject with all values BLQ through first 12 hours, 1/2 LOQ imputed for timepoints needed to calculate $AUC_{0\text{-}12}$.

[c] PK mean endpoint results presented as geometric means with 95% CI's; PD mean endpoint results presented as arithmetic means with 95% CI's.

[d] One subject discontinued ~16 hours after dosing; only $AUC_{0\text{-}12}$, GIR $AUC_{0\text{-}12}$, and onset of action were calculated for this subject.

[e] Analysis performed on log scale; results back transformed to original scale.

[f] Median (min, max).

[g] Determined from smoothed data.

Key design elements of these studies, including the single-dose crossover design and use of the euglycaemic clamp platform, are consistent with available regulatory guidance for the development of follow-on/biosimilar insulins[3] and multiple precedent studies assessing the pharmacology of basal insulins.[18–21] The 0.4 units/kg dose of MK-1293 and Lantus used in both studies permitted effective PK and PD characterization and comparison and is both clinically relevant and consistent with precedent from prior insulin glargine clamp studies.[18,19] Comparison of MK-1293 and Lantus PK and PD after single doses, as occurred in these studies, is supported by the absence of time-dependence of U100 insulin glargine PK[22] and the close temporal relationship between insulin PK and PD. The strength of the comparison of MK-1293 and Lantus was supported by rigourous comparisons in both T1D patients and healthy subjects, the use of a drug-specific LC-MS/MS PK assay, high quality euglycaemic clamp execution, and treatment comparisons on numerous endpoints characterizing PK and PD over the 24-hour insulin glargine dosing interval. In Study A, the use of a replicate design also permitted assessment of within-subject day-to-day PK and PD variability in T1D subjects.

Historically, insulin glargine PK has been assessed with non-specific insulin immunoassays. This has precluded rigourous PK assessment in T1D subjects because of interference from exogenous insulin, and has necessitated calculated adjustment for endogenous insulin in healthy subjects. However, insulin glargine PK can now be assessed without interference from other insulins using an LC-MS/MS-based approach.[7–9] After s.c. administration, parent insulin glargine is metabolized to its M1 and M2 metabolites.[23,24] Although all 3 have similar receptor-level potency,[25] M1 is the dominant circulating insulin glargine species after s.c dosing and is the principle mediator of therapeutic effects, while parent glargine and M2 circulate at very low to unquantifiable concentrations.[8,9] Therefore, PK comparison in these studies was based on LC-MS/MS-quantified M1 concentrations. In both studies, for all treatment comparisons, M1 PK profiles were closely aligned throughout the 30-hour (Study A) or 24-hour (Study B) assessment period, GMRs for primary (M1 $AUC_{0\text{-}24}$ and C_{max}) and secondary (M1 $AUC_{0\text{-}12}$ and $AUC_{12\text{-}24}$) PK endpoints were close to 1.0 (range: 0.92-1.02), and pre-specified similarity criteria (90% CIs of GMR between 0.80, 1.25) were met for both primary endpoints. Within-subject PK variability (% CV), assessed in Study A, was also similar between MK-1293 and Lantus.

The euglycaemic clamp platform is long established as the optimal approach for characterizing the PD profile of pharmacologic insulins and is recommended by the EMA for assessing pharmacologic similarity between insulin products.[3] Metrics characterizing accuracy and precision relative to clamp target glucose concentration confirmed high quality clamping in both studies. For both studies and all treatment comparisons, GIR profiles were closely aligned throughout the 24- to 30-hour assessment periods, arithmetic mean ratios for all primary GIR endpoints (GIR-$AUC_{0\text{-}24}$, GIR-$AUC_{0\text{-}12}$, GIR-$AUC_{12\text{-}24}$, GIR_{max}) were close to 1.0 (range: 0.90-1.03), and pre-specified similarity criteria were met for each of these primary endpoints. Consistent with PK, MK-1293 within-subject PD variability (% CV), assessed in Study A, was also similar to that of Lantus. The duration of action of MK-1293, assessed in T1D subjects in Study A, was also similar to that of Lantus and was greater than 30 hours in the majority of subjects.

Over the first 12 hours after dosing in both studies, M1 exposure ($AUC_{0\text{-}12}$) and PD effect (GIR-$AUC_{0\text{-}12}$) were slightly lower for MK-1293 compared to Lantus. However, as these differences were small (mean treatment ratios ≥0.90) and protocol-specified similarity criteria were met for all comparisons, these observations do not alter the conclusion of PK and PD similarity between MK-1293 and Lantus. Phase III studies in T1D[5] and T2D[6] subjects demonstrating equivalent HbA1c reduction at highly similar doses indicate that any minor PK and PD differences that may exist between MK-1293 and Lantus are not clinically impactful.

Overall, these studies have demonstrated a high degree of PK and PD similarity between MK-1293 and Lantus in both T1D patients and healthy subjects. Given the strong relationship between insulin pharmacology and glycaemic efficacy, these data provide evidence that the clinical efficacy profile of MK-1293 is similar to that of Lantus,[3] an expectation that is confirmed by the results of Phase III studies in T1D[5] and T2D[6] subjects.

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Conflict of interest

M. C., J. S. P., K. M. M., C. D. M., A. M. B., M. C. M. and Y. X. are or were employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, New Jersey and may hold stock and/or stock options in the company. E. W., L. M. and M. H. are or were employees of ProSciento, Inc., Chula Vista, California, which was funded by Merck & Co., Inc., Kenilworth, New Jersey, to conduct the 2 studies reported in this paper.

Author contributions

M. C. designed the studies, wrote the study protocols and wrote the manuscript. J. S. P. wrote the statistical analysis plans, performed the statistical analyses, contributed to the discussion and reviewed/edited the manuscript. K. M. M., C. D. H. and M. C. M. researched data and reviewed/edited the manuscript. Y. X. provided the pharmacokinetic data, contributed to the discussion and reviewed/edited the manuscript. A. M. B. performed the pharmacokinetic data analyses, contributed to the discussion and reviewed/edited the manuscript.

E. W., L. M. and M. H. conducted the studies, contributed to the discussion and reviewed/edited the manuscript. All of the authors are responsible for the work described in this manuscript. All authors were involved in at least 1 of the following: (1) conception, design, acquisition, analysis, statistical analysis or interpretation of data; (2) drafting the manuscript and/or revising it for important intellectual content. All authors provided final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPORTING INFORMATION

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