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Pharmacokinetic and pharmacodynamic bioequivalence of Gan & Lee insulin analogues aspart (rapilin®), lispro (prandilin®) and glargine (basalin®) with EU- und US-sourced reference insulins

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Abstract

Aim: For the successful approval and clinical prescription of insulin biosimilars, it is essential to show pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence to the respective reference products sourced from the European Union and the United States.

Methods: Three phase 1, randomized, double-blind, three-period crossover trials compared single doses of the proposed biosimilar insulin analogues aspart (GL-Asp, n = 36), lispro (GL-Lis, n = 38) and glargine (GL-Gla, n = 113), all manufactured by Gan & Lee pharmaceuticals, to the respective EU- and US-reference products in healthy male participants (GL-Asp and GL-Lis) or people with type 1 diabetes (GL-Gla). Study participants received 0.2 U/kg (aspart and lispro) or 0.5 U/kg (glargine) of each treatment under automated euglycaemic clamp conditions. The clamp duration was 12 h (aspart and lispro) or 30 h (glargine). Primary PK endpoints were the total area under the PK curves ($AUC_{ins.total}$) and maximum insulin concentrations ($C_{ins.max}$). Primary PD endpoints were the total area under the glucose infusion rate curve ($AUC_{GIR.total}$) and maximum glucose infusion rate (GIR_{max}).

Results: Bioequivalence to both EU- and US-reference products were shown for all three GL insulins. Least squares mean ratios for the primary PK/PD endpoints were close to 100%, and both 90% and 95% confidence intervals were within 80%–125% in all three studies. There were no noticeable differences in the safety profiles between test and reference insulins, and no serious adverse events were reported for the GL insulins.

Conclusion: GL-Asp, GL-Lis and GL-Gla are bioequivalent to their EU- and US-reference products.

KEYWORDS
bioequivalence, insulin aspart, insulin glargine, insulin lispro, pharmacodynamics, pharmacokinetics

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1 | INTRODUCTION

Insulin therapy is still a major cornerstone of diabetes therapy, essential in people with type 1 diabetes and advanced type 2 diabetes.[1] Insulin analogues have become the dominant insulin therapy in clinical practice since the start of their development in the 1990s,[1,2] as they are associated with improved postprandial glucose levels (short-acting insulin analogues) and lower fasting hyperglycaemia (basal insulin analogues). However, their high costs have been a significant barrier to insulin accessibility in many low-income and middle-income countries, and led to half of the people who needed insulin but lacked access.[3] Even in the United States, insulin rationing due to affordability issues occurs in as many as 20% of patients leading to a high risk of ketoacidosis and a potentially lethal outcome.[4–6] There is, therefore, a significant unmet need for more affordable insulins. One potential solution might be insulin biosimilars, which are as effective and safe as commercially available reference insulins, but at lower cost.

Gan & Lee Pharmaceuticals is an insulin manufacturer headquartered in China, with a branch in New Jersey in the United States, which, like many other companies, aims at developing insulin biosimilars, including insulin aspart (GL-Asp), insulin lispro (GL-Lis) and insulin glargine (GL-Gla). All these proposed insulin biosimilars have already been marketed in China since 2020 (GL-Asp), 2007 (GL-Lis) and 2005 (GL-Gla), respectively. They are all produced by recombinant DNA technology in full compliance with the high standards of Good Manufacturing Practice; they also share an identical primary and advanced structure to commercially available formulations of these analogues (e.g. NovoLog®/NovoRapid®, Humalog®, Lantus®). We conducted three phase 1 bioequivalence studies (one for each analogue) to investigate the bioequivalence of the GL insulin analogues to their respective reference insulins commercially available in Europe (EU-Asp; EU-Lis; EU-Gla) and the United States (US-Asp; US-Lis; US-Gla). Proof of both pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence is required to obtain marketing authorization in each market[7,8]; therefore, these studies were done in accordance with regulatory guidelines issued by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA).[9,10]

2 | METHODS

2.1 | Study design

Three phase 1, randomized, double-blind, three-period crossover trials compared single doses of the proposed biosimilar insulin analogues GL-Asp (n = 36), GL-Lis (n = 38) and GL-Gla (n = 113), all manufactured by Gan & Lee Pharmaceuticals, with respective EU- and US-reference products in healthy male subjects (GL-Asp and GL-Lis) or people with type 1 diabetes (GL-Gla) (Figure 1). The main inclusion/exclusion criteria are listed in the Supplementary Information: Data S1. Written informed consent was obtained from all participants, and the studies were conducted in accordance with the principles of Good Clinical Practice as defined by the International Conference of Harmonization (ICH).

The studies consisted of several visits, including an information visit to obtain informed consent, a screening visit to assess eligibility for participation, three dosing visits with a wash-out period between each visit, and a follow-up visit after the last dose. The insulin products were administered in a randomized order using an automated 12 h (aspart and lispro) or 30 h (glargine) euglycaemic glucose clamp at target blood glucose concentrations of 81 mg/dL (aspart and lispro) or 100 mg/dL (glargine). The euglycaemic glucose clamp was conducted using the ClampArt® device (Profil), which continuously monitored the subject's blood glucose and administered glucose infusion rates (GIR) to maintain blood glucose close to the target blood glucose concentration. The GIR was calculated every minute based on the aggregate blood glucose values recorded by the device. The glucose clamp device automatically adjusted the infused GIR to maintain the target blood glucose concentration based on the actual measured blood glucose concentration and the degree of variability in the previous minute. More details about clamp methodology are provided in the Supplementary Information: Data S1.

Blood samples were collected at pre-specified intervals before and up to 12 h (insulin aspart and insulin lispro) or 30 h (insulin glargine) after dosing for measurement of blood glucose, plasma insulin and C-peptide. For insulin glargine, blood samples were also analysed for the active metabolites 1 and 2 of glargine (M1 and M2).

2.2 | Study endpoints

The primary PK endpoints were the total area under the insulin concentration curve ($AUC_{ins.total}$) and maximum observed insulin concentration ($C_{ins.max}$). The primary PD endpoints were the total area under the GIR curve ($AUC_{GIR.total}$) and maximum GIR (GIR_{max}). Safety endpoints included adverse events (AEs), serious AEs (SAEs), laboratory safety, physical examinations, vital signs, electrocardiograms and local tolerability at the injection site.

2.3 | Assessments

PK samples were collected and analysed using a validated mass spectrometry immunoassay-liquid chromatography-mass spectrometry/mass spectrometry. The lower limit of quantification of the method was 70 pg/mL, and the upper limit of quantification was 5000 pg/mL.

Safety assessments included continuous monitoring of vital signs and electrocardiograms, laboratory safety parameters, determination of anti-insulin antibodies, physical examination, documentation of AEs and evaluation of local tolerability.

2.4 | Statistical analyses

Statistical analyses were performed using SAS® (version 9.4; SAS Institute Inc.) and WinNonlin® (version 8.1; Certara L.P.). Analyses were performed for the per-protocol set (PPS) and the modified PPS

for PK, which included all randomized subjects who completed the trial without a major deviation affecting PK and all profiles that had a baseline value of ≤5% of $C_{ins.max}$ and had <50% of post-dose concentration values below the lower limit of quantification.

The primary PK endpoints were logarithmically transformed and analysed using a mixed-effects model analysis of variance (ANOVA) model with sequence, period and investigational medicinal product (IMP) as fixed effects and subject within the sequence as a random effect. The least squares (LS) mean of each IMP and the ratio of test to reference insulins were estimated together with the corresponding 90% confidence intervals (CIs). The estimates and upper and lower bounds of the 90% CIs were then exponentially transformed and multiplied by 100% to obtain the estimated LS-mean response ratio between the IMPs. If the 90% CIs of the estimated ratio of $AUC_{ins.total}$ and $C_{ins.max}$ fell within the limits of 80%-125%, PK equivalence would be concluded.

The same ANOVA model was used to analyse the primary PD endpoints, but the PD endpoints were used without logarithmic transformation. Instead, the LS-mean of each IMP was estimated, and the ratios of the LS-mean (test/reference insulin) were determined. Fieller's Theorem was used to calculate the 90% and 95% CIs of the LS-mean ratio. Bioequivalence was assumed if the 90% (comparison with the US reference) or 95% (comparison with the EU reference) CIs fell within the limits of 80%-120%. In addition, a sensitivity analysis of glargine was performed with log-transformed data and excluding data of low responders (the definition of low responders was pre-specified as subjects showing $AUC_{GIR.0\text{-}24h}$ values with the reference treatments that were <5% of the observed geometric LS-mean $AUC_{GIR.0\text{-}24h}$ for the reference treatment of the remaining subjects) (ANOVA, limits: 80.00%-125.00%). GIR profiles were smoothed using a locally weighted regression technique for the calculation of time-related PD endpoints and GIR_{max} using smoothing parameters of 0.1 (aspart and lispro studies) or 0.3 (glargine study).

The secondary PK and PD AUC endpoints were compared using the same statistical approach as for the primary PK or PD endpoints but did not have to fulfil any equivalence criteria, and the secondary PK/PD endpoints $t_{ins.max}$ and $t_{GIR.max}$ were analysed by non-parametric methods using the Wilcoxon signed rank test and Hodges and Lehmann estimates.

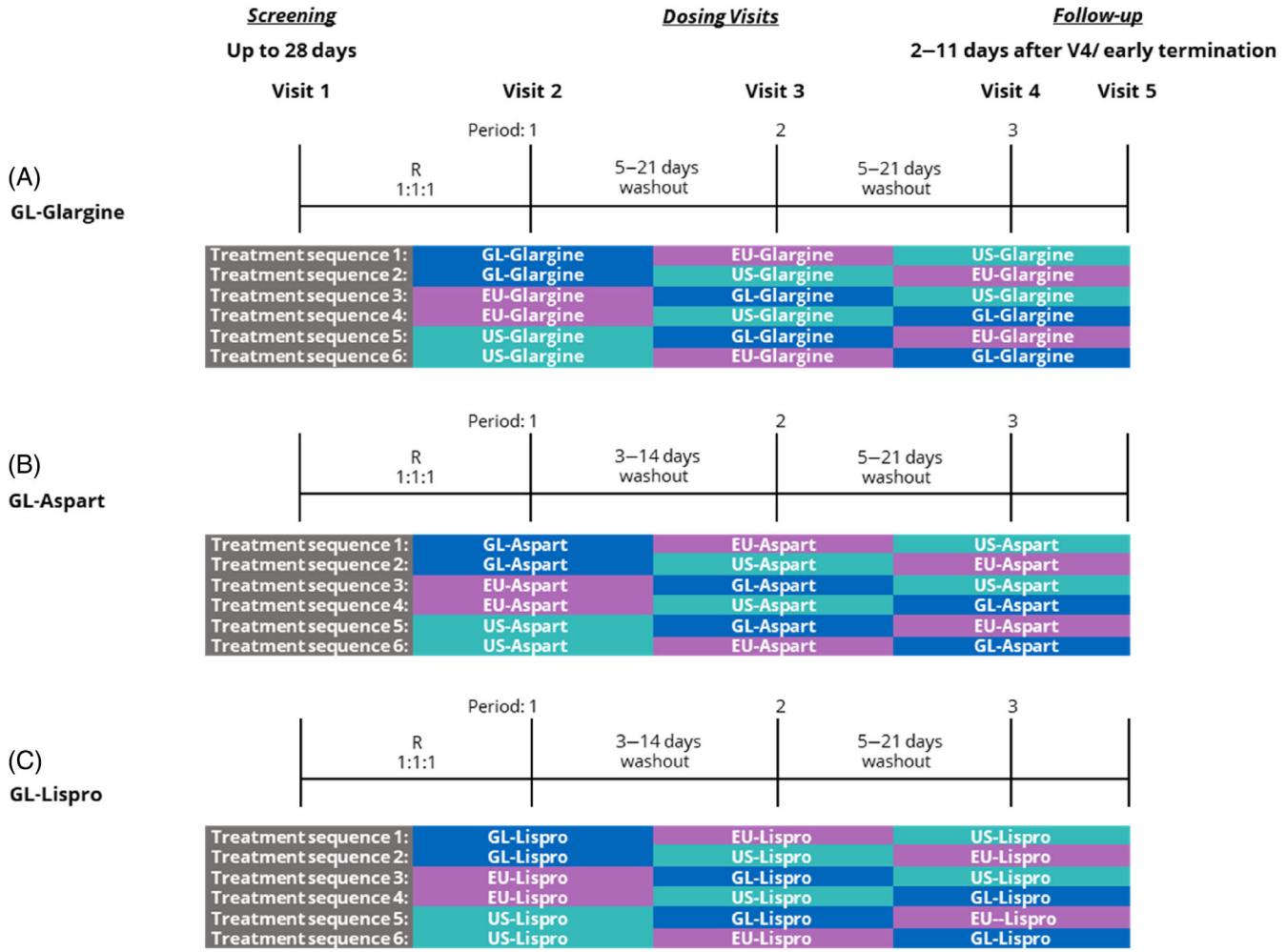

FIGURE 1 Schematic overview of the chronological structure of the trials: (A) Gan & Lee insulin analogue (GL)-Glargine; (B) GL-Aspart; and (C) GL-Lispro.

Safety endpoints were analysed based on the safety analysis set including all subjects receiving at least one dose of the study medication. Safety results are presented using descriptive statistics by visit/treatment.

3 | RESULTS

3.1 | Subject disposition, and demographic and baseline characteristics

Subject disposition for all three studies is given in CONSORT diagrams (Figure S1). Demographic and baseline characteristics are summarized in Table 1.

3.2 | Pharmacokinetics

Mean PK/PD profiles for the three studies are summarized in Figure 2 and the primary PK and PD endpoints are given in Tables 2 and 3. In general, the mean PK profiles of the three insulins compared in one study were similar with only small differences in the mean maximum concentrations. The primary PK endpoints were similar across the three insulins in each study as indicated by most ratio point estimates being close to 100% for both the total AUC and C_{max} of the insulin aspart, insulin lispro and M1 concentrations (the predominant metabolite of insulin glargine). While there were a few exceptions with 10% differences in C_{max} (GL-Lis vs. US-Lis), bioequivalence criteria (90% CI of the geometric LS-mean treatment

TABLE 1 Demographic and baseline characteristics of the subjects

Parameter, unit	Aspart Arithmetic mean ± SD (N = 36)	Lispro Arithmetic mean ± SD (N = 38)	Glargine Arithmetic mean ± SD (N = 113)
Age, years	36.2 ± 11.78	41.1 ± 14.63	42.4 ± 12.05
Height, cm	180.3 ± 7.34	179.5 ± 6.66	180.5 ± 7.28
Weight, kg	79.3 ± 12.22	78.6 ± 10.44	84.1 ± 10.26
Body mass index, kg/m^2	24.3 ± 2.72	24.3 ± 2.84	25.7 ± 2.03
Fasting plasma glucose, mmol/L	5.0 ± 0.35	5.1 ± 0.26	NA

Abbreviations: N, number of subjects; NA, not available; SD, standard deviation.

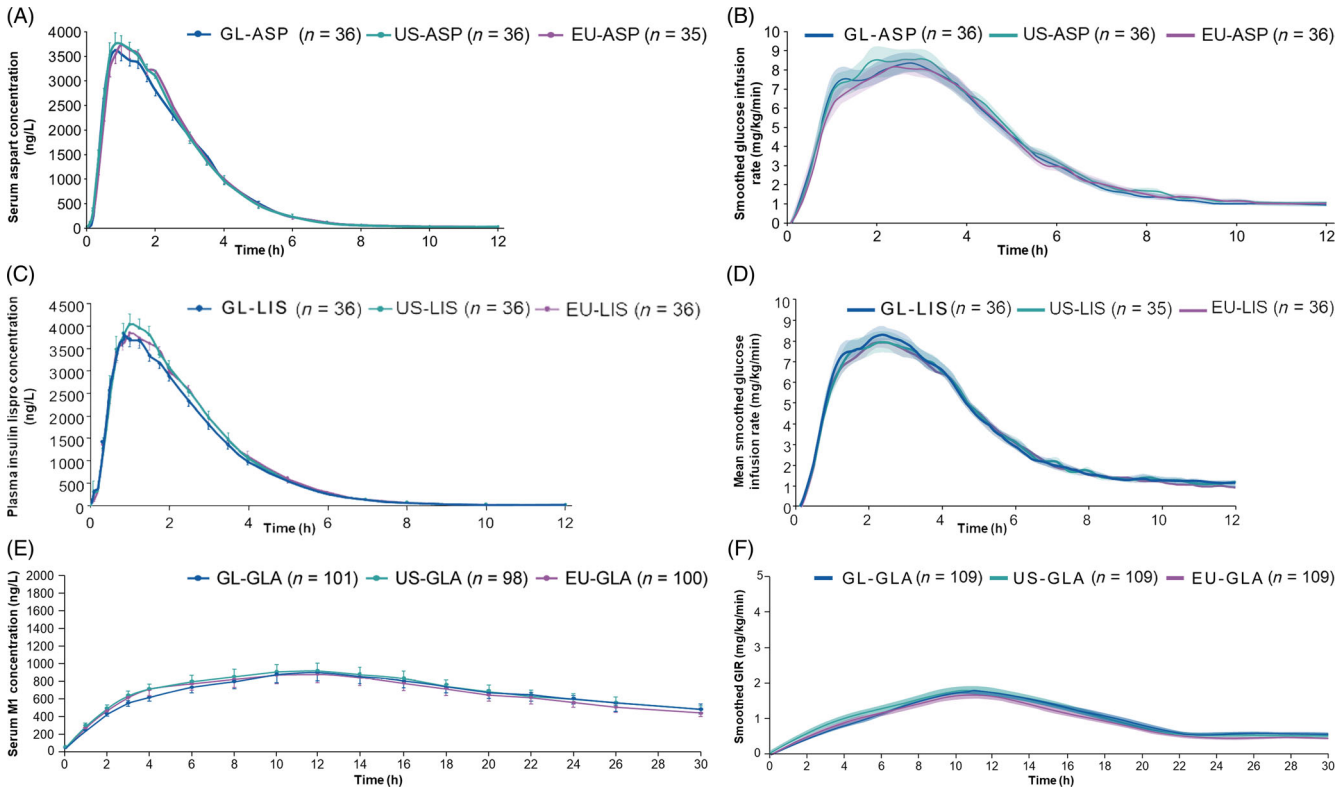

FIGURE 2 Pharmacokinetic and pharmacodynamic profiles of the GL-insulins and their reference insulins. (A) Insulin aspart (ASP) concentration-time profiles after s.c. administration of GL-ASP, EU-ASP or US-ASP (0.2 U/kg); (B) smoothed glucose infusion rate (GIR) profiles of the three aspart preparations; (C) insulin lispro (LIS) concentration-time profiles after s.c. administration of GL-LIS, EU-LIS and US-LIS (0.2 U/kg); (D) smoothed GIR profiles of the three lispro preparations; (E) insulin glargine (GLA) concentration-time profiles after s.c. administration of GL-GLA, EU-GLA and US-GLA (0.5 U/kg); (F) smoothed GIR profiles of the three GLA preparations.

ratio within 80.00%-125.00%) were met for the primary PK endpoints for all three GL proposed insulin biosimilar formulations versus their respective comparators from the European Union and the United States (Table 2). PK bioequivalence was therefore established between the treatments. Likewise, descriptive statistics for the secondary PK endpoints showed similar results for GL insulins and the comparator insulins (Tables S1-S3). In healthy subjects, mean C-peptide concentrations decreased after dosing and increased from 2 h post-dosing to slightly higher values than at baseline at 8 h post-dosing, reflecting the suppressive effect of the insulin administration on endogenous insulin secretion. There was no marked difference in the mean C-peptide profiles between GL-Asp and GL-Lis with their reference insulins (Figure S2).

3.3 | Pharmacodynamics

In general, PD results based on GIRs during the clamp experiments, followed the PK outcomes with similar mean profiles between the three insulin preparations in each study (Figure 2). Ratio point estimates between the GL insulins and the respective comparators from both the European Union and the United States ranged from 96% to 104% for the short-acting analogues and from 95% to 107% for insulin glargine. As expected, variability was larger for PD than for PK resulting in wider CIs, in particular for insulin glargine, which is known to have a large inter- and intra-subject variability.[11] Nevertheless, the results of the Fieller's Theorem analysis showed that the equivalence limits (95% CI of the LS-mean treatment ratio of the untransformed data within the limits of 80.00%-120.00%) were met for the two primary PD endpoints for all three GL insulins in their comparison with their EU and US comparators (Table 3). The secondary PD analysis using log-transformed data confirmed the PD bioequivalence of the GL insulins with their comparators.

In addition, there were only small differences between GL insulins and the respective comparative insulins in the secondary PD endpoints, which were in the same magnitude or smaller as the differences between the two comparator insulins themselves (Tables S1-S3). Clamp quality (assessed as described previously[12]) showed coefficients of variation at about 4% (glargine) or 5% (lispro, aspart) and mean deviations from target as small as 0.5-1 mg/dl (Table 4).

3.4 | Safety

Safety data were comparable between the GL proposed insulin biosimilars and the comparator insulins (Tables S4-S6). Drug-related AEs that were possibly or probably related to the study insulins occurred

TABLE 2 Primary pharmacokinetic parameters of the three studies

Aspart					
Parameter, unit	**GL-Asp (N = 36)**	**EU NovoRapid (N = 35)**	**US NovoLog (N = 36)**	**Geometric LS-mean ratio % (90% CI) GL-Asp versus EU NovoRapid**	**Geometric LS-mean ratio % (90% CI) GL-Asp versus US NovoLog**
$AUC_{ins\ 0-12h}$, h*ng/L	10697.8 ± 1999.24	10999.4 ± 2111.19	10924.2 ± 1801.64	97.08 (94.50, 99.73)	97.53 (94.97, 100.16)
$C_{ins.max}$, ng/L	4103.1 ± 950.51	4197.4 ± 1049.62	4287.5 ± 1115.57	97.74 (91.90, 103.94)	96.43 (90.73, 102.49)
Lispro					
Parameter, unit	**GL-Lis (N = 36)**	**EU Humalog (N = 36)**	**US Humalog (N = 36)**	**Geometric LS-mean ratio % (90% CI) GL-Lis versus EU Humalog**	**Geometric LS-mean ratio % (90% CI) GL-Lis versus US Humalog**
$AUC_{ins\ 0-12h}$, h*ng/L	10957.7 ± 1545.74	11576.3 ± 2147.68	11684.5 ± 2283.31	95.29 (92.47, 98.20)	94.59 (91.79, 97.48)
$C_{ins.max}$, ng/L	4229.2 ± 1292.71	4331.9 ± 1252.70	4775.0 ± 1796.00	97.04 (89.98, 104.64)	89.79 (83.26, 96.83)
Glargine					
Parameter, unit	**GL-Gla (N = 101)**	**EU Lantus (N = 100)**	**US Lanus (N = 98)**	**Geometric LS-mean ratio % (90% CI) GL Lantus versus EU Lantus (N = 95)**	**Geometric LS-mean ratio % (90% CI) GL Lantus versus US Lantus (N = 95)**
$AUC_{ins\ 0-24h}$, h*ng/L	16977.3 ± 16419.80	17010.9 ± 18330.50	17625.1 ± 18818.27	102.45 (97.52; 107.62)	98.36 (93.71; 103.25)
$C_{ins.max}$, ng/L	972.5 ± 952.65	979.2 ± 1060.21	1001.9 ± 1052.49	101.52 (96.04; 107.31)	98.58 (93.38; 104.06)

Note: Data are presented as mean ± standard deviation.

Abbreviations: $AUC_{ins.0-12h}$, area under the insulin concentration curve from 0 to 12 h; $AUC_{ins.0-24h}$, area under the insulin concentration curve from 0 to 24 h; $C_{ins.max}$, maximum observed insulin concentration; CI, confidence interval; GL-Asp, Gan & Lee insulin analogue aspart; GL-Gla, Gan & Lee insulin analogue glargine; GL-Lis Gan & Lee insulin analogue lispro; LS, least square; N, number of subjects.

TABLE 3 Primary pharmacodynamic parameters of the three studies

Aspart					
Parameter, unit	**GL-Asp (N = 36)**	**EU NovoRapid (N = 36)**	**US NovoLog (N = 36)**	**Arithmetic LS-mean ratio % (95% CI) GL-Asp versus EU NovoRapid**	**Arithmetic LS-mean ratio % (90% CI) GL-Asp versus US NovoLog**
$AUC_{GIR.0-12h}$, mg/kg	2638.7 ± 912.97	2598.1 ± 753.22	2737.4 ± 1044.14	101.56 (96.88, 106.26)	96.39 (92.50, 100.55)
GIR_{max}, mg/kg/min	9.969 ± 3.3641	9.589 ± 3.1582	10.239 ± 4.6400	103.97 (98.38, 109.81)	97.36 (91.45, 104.00)
Lispro					
Parameter, unit	**GL-Lis (N = 36)**	**EU Humalog (N = 36)**	**US Humalog (N = 35)**	**Arithmetic LS-mean ratio % (95% CI) GL-Lis versus EU Humalog**	**Arithmetic LS-mean ratio % (90% CI) GL-Lis versus US Humalog**
$AUC_{GIR.0-12h}$, mg/kg	2633.2 ± 812.70	2549.1 ± 678.67	2562.6 ± 760.73	103.30 (97.25, 109.57)	101.48 (96.81, 106.33)
GIR_{max}, mg/kg/min	9.495 ± 3.1611	9.169 ± 2.9266	9.364 ± 3.4496	103.55 (97.37, 110.07)	100.75 (95.62, 106.26)
Glargine					
Parameter, unit	**GL-Gla (N = 109)**	**EU Lantus (N = 109)**	**US Lanus (N = 109)**	**Arithmetic LS-mean ratio % (95% CI) GL Lantus versus EU Lantus (N = 108)**	**Arithmetic LS-mean ratio % (90% CI) GL Lantus versus US Lantus (N = 108)**
$AUC_{GIR.0-24h}$, mg/kg	1524.6 ± 1043.53	1426.2 ± 1035.67	1584.3 ± 961.31	106.63 (96.24;118.35)	95.29 (87.34;103.70)
GIR_{max}, mg/kg/min	1.920 ± 1.2204	1.789 ± 1.1460	2.008 ± 1.1930	107.17 (96.00;119.68)	94.90 (86.09;104.48)

Note: Data are presented as mean ± standard deviation.
Abbreviations: $AUC_{GIR.0-12h}$, area under the glucose infusion rate curve from 0 to 12 h; $AUC_{GIR.0-24h}$, area under the glucose infusion rate curve from 0 to 24 h; CI, confidence interval; GIR_{max}, maximum glucose infusion rate; GL-Asp, Gan & Lee insulin analogue aspart; GL-Gla, Gan & Lee insulin analogue glargine; GL-Lis, Gan & Lee insulin analogue lispro; LS, least square; N, number of subjects.

TABLE 4 Clamp quality parameters

	GL insulin	EU reference	US reference
Lispro study			
Precision, coefficient of variation %	4.65 ± 1.37	5.16 ± 1.48	5.00 ± 1.77
Control deviation, deviation from target; mg/dL	0.15 ± 0.35	0.11 ± 0.30	0.32 ± 0.41
Aspart study			
Precision, coefficient of variation %	5.12 ± 1.45	4.86 ± 1.48	5.44 ± 2.34
Control deviation, deviation from target; mg/dL	0.48 ± 0.40	0.33 ± 0.41	0.55 ± 0.84
Glargine study			
Precision, coefficient of variation %	3.92 ± 1.82	3.95 ± 1.67	4.19 ± 1.77
Control deviation, deviation from target; mg/dL	1.08 ± 2.00	0.98 ± 1.28	1.09 ± 1.74

Note: Data are presented as means ± SDs.

in seven people following the administration of GL-Asp versus six to seven participants with the comparator insulin aspart formulations and in four with GL-Lis compared with three to eight with the comparator insulins. Twenty-four treatment-emergent AEs (TEAEs) occurred in 21 subjects with GL-Gla versus 39 events, respectively, with the glargine comparator insulins from the European Union and

the United States. All reported TEAEs resolved or recovered. One SAE occurred after EU-Gla administration (hospitalization because of generalized tonic-clonic seizure without hypoglycaemia) and resulted in the participant's discontinuation from the trial and unblinding for regulatory reporting.

Injection site reactions were rare (one mild erythema lasting for 1 h after the injection of GL-Asp, one mild and transient injection site reaction after injection of US Humalog, and five mild and transient injection site reactions after injection of the insulin glargine formulations (one with GL-Gla, two EU Lantus and two US Lantus).

4 | DISCUSSION

Proof of biosimilarity (including PK/PD equivalence) is required to obtain marketing authorization in the respective markets (European Union and United States). The results showed that GL-Asp, GL-Lis and GL-Gla are equivalent to their EU- and US-reference products, as the 90% CIs of the respective geometric LS-mean treatment ratio lay within the limits of 80.00% and 125.00%, based on the primary PK endpoints $AUC_{ins.total}$ and $C_{ins.max}$. The LS-mean ratios for the primary PK/PD endpoints were close to 100%, and both 90% and 95% CIs were within 80%-125% in all three studies. There were no notable differences in the safety profiles between the test and reference insulins, and no SAEs related to either of the GL insulins were reported.

Key design elements of these studies, including a single-dose crossover design, were in line with the available guidelines for the development of biosimilar insulins. A single-dose crossover design was chosen because each person serves as their own control allowing intra-subject comparisons between formulations and removing inter-subject variability from the comparison. Randomization and a double-blind design were used to avoid observer and subject bias.

The euglycaemic clamp technique is widely used to assess insulin bioactivity and is recommended by many regulatory authorities, including the FDA and EMA for showing PD biosimilarity between insulins in clinical pharmacology studies. The clamp setting was based on an automated glucose clamp technique with continuous blood glucose measurements and minute-by-minute adaptations of GIRs to achieve the highest clamp quality possible while also reducing potential investigator-related bias.[13] Moreover, the euglycaemic clamp technique minimized the risk of any drug-induced hypoglycaemia. Per EMA guidance, a population of healthy subjects was selected for aspart and lispro trials as they represent an appropriate insulin-sensitive population for the study of short-acting insulins and have lower intra-individual variability compared with patients with type 1 diabetes mellitus. In contrast, patients with type 1 diabetes mellitus were selected for investigating GL-Gla not only to provide data relevant to real-world populations who use insulin glargine but also because these patients lack endogenous insulin, allowing a robust insulin's time-action profile without the confounder of competing endogenous insulin, which is particularly important for the late phase of the clamp and for duration of action.[13,14] In addition, clamp experts concur that PD outcomes, particularly towards the end of clamp procedures, can be influenced by endogenous insulin secretion during glucose clamp tests in both healthy individuals and those with type 2 diabetes mellitus.[15]

To avoid any confounding effect of endogenous insulin concentrations on PK determinations in the studies with healthy subjects, insulin aspart and insulin lispro concentrations for the PK analysis were determined using a method specific for this analogue. Methods such as selecting insulin doses at the higher end of the range (0.2 U/kg) and a clamp target at the lower end of the euglycemic range were chosen as they would probably facilitate suppression of endogenous insulin throughout the clamp.[13] In addition, C-peptide, which indicates an equal amount of endogenous insulin secreted into the blood, was measured in parallel with insulin concentrations to assess the level of the suppression of endogenous secretion during the clamp study and to identify subjects whose endogenous insulin production could have potentially interfered with PD measurements. Mean C-peptide concentrations reflecting endogenous insulin secretion were successfully suppressed after the tested insulin administration, and the suppression on mean C-peptide profiles was similar between GL-Asp and GL-Lis with their reference insulins. Likewise, there was no evidence of any confounding effects of differences in clamp quality on PD results. The results for both precision and deviation from the target showed that the clamp performances were comparable across the tested IMPs and as good or better as reported for PK/PD studies with other biosimilar insulins.[16,17]

While the results of the primary PD analyses were robust and PD bioequivalence was supported by the secondary PD endpoints, further analyses were performed to look into some peculiarities of the insulins investigated. One of the difficulties in assessing bioequivalence of insulin glargine preparations is the occurrence of low responders that show little or no GIR even after high doses of insulin glargine administration.[13,16] Indeed, we also observed low responders in our study defined as clamps where the geometric LS means of $AUC_{GIR.0\text{-}24h}$ of the reference treatment was <5% of the observed geometric mean $AUC_{GIR.0\text{-}24h}$ of the remaining subjects for the reference treatment. Overall, there were six clamps that fulfilled the low-responder definition or had no GIRs (none with GL-Gla, four EU-Gla, two US-Gla). As these low responders introduce a lot of variability to PD endpoints in the conventional bioequivalence analyses with geometric means and log-transformed data, the primary analysis in the glargine study was based on arithmetic means and untransformed data. Nevertheless, a sensitivity analysis with log-transformed data confirmed bioequivalence between GL-Gla and the two reference insulins. Likewise, analyses/or excluding profiles based on pre-defined criteria (e.g. C-peptide limits, hypoglycaemic events) showed bioequivalence for the GL insulins and the reference insulins in all three studies.

No new or unexpected safety findings were observed in these three studies, and all IMPs were well-tolerated, with headache being the most common AE and observed in all treatment groups. Nevertheless, headache is common in the experimental setting of a euglycaemic clamp with prolonged fasting and bed rest periods. No drug-

related SAEs were reported for GL insulins and none of the TEAEs for GL insulins resulted in a discontinuation of IMP. There was no signal for local tolerability issues with GL insulins. Single dose glucose clamp studies only appear to have limited power to show safety signals of new insulins; however, the proposed biosimilar insulins show identical primary and similar secondary and tertiary molecular structures as the reference insulins. New safety signals are therefore not expected, and regulatory agencies such as FDA and EMA have therefore potentially waived the requirement of larger clinical studies for biosimilar insulins provided that biosimilarity can be convincingly concluded from the physicochemical and functional characterization and from single-dose PK/PD studies.[9,10] Even immunogenicity data from longer-term clinical studies may no longer need to be provided as there is minimal or no clinical relevance of immunogenicity with insulin product use.[10] Nevertheless, clinical studies with a treatment duration of up to 26 weeks were done with GL-Gla in comparison with commercially available reference insulin glargine preparations in people with type 1 and 2 diabetes (NCT03371082/NCT03371108). These studies did not identify new safety concerns versus the reference insulins with non-severe hypoglycaemia being the most frequent TEAEs in all study arms (without significant differences across treatments).

5 | CONCLUSION

Overall, the results of these trials showed that three insulins show PK and PD bioequivalence to reference insulins in human subjects. In line with regulatory guidelines on biosimilar insulins,[9,10] these results support the use of GL-Asp, GL-Lis and GL-Gla as an efficacious alternative to the currently available original insulin analogues.

AUTHOR CONTRIBUTIONS

LPM, GA, EZ and TH contributed to data analysis and study conduct. WC, AH, TX, LL, TH and CH contributed to writing and editing of the manuscript. WC, JL, ZG and TH contributed to the project management of the study. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15281.

DATA AVAILABILITY STATEMENT

The data that support the findings of these studies are available on request from the corresponding author Zhongru Gan. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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