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Pharmacokinetic and pharmacodynamic bioequivalence of proposed biosimilar MYL-1501D with US and European insulin glargine formulations in patients with type 1 diabetes mellitus

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Abstract

Aims: To report phase 1 bioequivalence results comparing MYL-1501D, US reference insulin glargine (US IG), and European reference insulin glargine (EU IG).

Materials and methods: The double-blind, randomized, three-way crossover study compared the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of MYL-1501D, US IG and EU IG. In total, 114 patients with type 1 diabetes (T1DM) received 0.4 U/kg of each study treatment under automated euglycaemic clamp conditions. Insulin metabolite M1 concentrations, insulin glargine (IG) and glucose infusion rates (GIRs) were assessed over 30 hours. Primary PK endpoints were area under the serum IG concentration–time curve from 0 to 30 hours ($AUC_{ins.0-30h}$) and maximum serum IG concentration ($C_{ins.max}$). Primary PD endpoints were area under the GIR–time curve from 0 to 30 hours ($AUC_{GIR0-30h}$) and maximum GIR (GIR_{max}).

Results: Bioequivalence among MYL-1501D, US IG and EU IG was demonstrated for the primary PK and PD endpoints. Least squares mean ratios were close to 1, and 90% confidence intervals were within 0.80 to 1.25. The PD GIR–time profiles were nearly superimposable. There were no noticeable differences in the safety profiles of the three treatments, and no serious adverse events were reported.

Conclusions: Equivalence with regard to PK and PD characteristics was shown among MYL-1501D, US IG and EU IG in patients with T1DM, and each treatment was well tolerated and safe.

KEYWORDS

bioequivalence, biosimilar, diabetes, insulin, insulin glargine, pharmacokinetics, pharmacodynamics, phase 1, type 1 diabetes mellitus

1 | INTRODUCTION

For patients with type 1 diabetes mellitus (T1DM), insulin therapy is the mainstay of treatment.[1] Patients with T1DM typically use both prandial (mealtime) insulin and long-acting basal insulin analogues.[1] Long-acting insulins, such as insulin glargine (IG), are efficacious and have demonstrated safety benefits over NPH insulin, mainly a lower incidence of nocturnal hypoglycaemic events[2,3]; however, the cost of these agents is often a factor in patients' access to therapy.[4] Additionally, high insulin costs have been shown to have deleterious effects on patient adherence and thus glycaemic control.[5,6] In this respect, basal insulin is like other biological medications, for which costs frequently limit patient access across the globe.[7]

Biosimilars to insulins may be an option to help reduce costs and improve access to treatment.[7,8] The pathway for approval of biosimilars in the United States by the US Food and Drug Administration was in part provided by the Biologics Price Competition and Innovation Act.[9] An abbreviated approval pathway under the US Federal Food, Drug and Cosmetic Act is used for approval of insulin products, which are considered a follow-on biologic.[10] The European Medicines Agency (EMA) also published guidelines for the development of biosimilar insulins, which require demonstration of pharmacokinetic (PK) and pharmacodynamic (PD) equivalence.[11] As patents for biologics expire, use of biosimilars or follow-on biologics for therapies such as insulin may increase as high-quality alternatives become available.[7]

Lantus® (insulin glargine injection; Sanofi-Aventis US LLC, Bridgewater, New Jersey) is a long-acting insulin analogue indicated for once-daily subcutaneous administration in patients with T1DM.[12] MYL-1501D (developed jointly by Mylan Inc., Canonsburg, Pennsylvania, and Biocon Ltd, Bangalore, India) is a proposed biosimilar to US and European formulations of IG (US IG and EU IG, respectively). The objective of the present study was to evaluate the bioequivalence of PK and PD characteristics of MYL-1501D with both US IG and EU IG.

2 | MATERIALS AND METHODS

2.1 | Study design

GLARGCT100111 was a single-centre, randomized, double-blind, single-dose, three-way crossover, euglycaemic clamp phase 1 study that was conducted from November 8, 2011 to March 7, 2012 (Clinical trial registration: EudraCT, 2011–003563-30). Eligible patients were aged 18 to 55 years. Patients must have been clinically diagnosed with T1DM for ≥1 year, were required to have fasting serum C-peptide levels ≤0.3 pmol/mL, and were otherwise generally healthy non-smokers. Additional inclusion criteria were body mass index between 18.5 and 29.9 kg/m^2, stable insulin treatment for ≥6 months before screening, glycated hemoglobin (HbA1c) levels ≤75 mmol/mol (≤9.0%), and stable weight with no more than a 5-kg gain or loss within 3 months of screening. Key exclusion criteria included insulin resistance (defined as requiring ≥1.4 U/kg/d insulin), use of glucocorticoids or other drugs that affect glycaemic control within the last 6 months, or use of blood glucose-lowering drugs other than insulin or insulin analogues. Patients were required to give written informed consent. The trial was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice guidelines and all local and federal laws and regulations, and was approved by an ethics committee and the Federal Institute for Drugs and Medical Devices (*Bundesinstitut für Arzneimittel und Medizinprodukte*).

Patients were randomly allocated to receive a single subcutaneous injection of MYL-1501D, US IG or EU IG at a dose of 0.4 U/kg on three separate dosing visits (Figure S1). Patients did not receive the same treatment twice (six possible sequences). Visits were spaced 5 to 28 days apart to include a minimum 5-day resting period, ensuring an adequate washout period from previous dosing to avoid any carryover effects. To ensure washout of previous insulin therapy, patients taking insulin injections usually administered the last basal analogue injection in the morning 2 days before the clamp. On the morning of the next day (1 day before the clamp), they used NPH insulin and continued prandial insulins (human or analogue) until bedtime the night before the clamp. In case the duration of action of NPH was too short to last the entire night, patients were instructed to measure their blood glucose between 2:00 AM and 3:00 AM and, if it was high, they could inject up to 8 U of prandial insulin (human or analogue).

Each visit included a 30-hour automated euglycaemic clamp procedure (Biostator®; MTB Medizintechnik, Amstetten, Germany) preceded by a 1- to 6-hour baseline period for stabilization of blood glucose levels to the clamp target level (5.5 mmol/L [100 mg/dL]). During the baseline stabilization period, a variable infusion of human regular soluble insulin or glucose was initiated to obtain a blood glucose level of 5.5 (± 20%) mmol/L (100 mg/dL). This level had to be kept continuously for at least 1 hour before trial product administration. From 1 hour before trial product administration, the insulin infusion rate (if any) was decreased as much as possible while ensuring that no glucose was infused. At 20 minutes before trial product administration, the insulin infusion was completely terminated. If the target blood glucose level could not be established by 6 hours, the visit was terminated and the patient could be scheduled for a new dosing visit 1 to 28 days later. The Biostator recorded blood glucose concentrations and glucose infusion rates (GIRs) at 1-minute intervals for PD evaluation. The device's blood glucose measurements were double-checked and adjusted, if necessary, at regular intervals (at least every 30 minutes) by parallel measurements (Super GL Glucose Analyser, Hitado Diagnostic Systems, Möhnesee, Germany).

2.2 | Study endpoints

The primary PK endpoints were area under the serum IG concentration–time curve from 0 to 30 hours ($AUC_{ins.0–30h}$) and maximum serum IG concentrations ($C_{ins.max}$). The primary PD endpoints were area under the GIR–time curve from 0 to 30 hours ($AUC_{GIR0–30h}$) and maximum GIR (GIR_{max}). Secondary PK endpoints included AUC from 0 to 6 hours ($AUC_{ins.0–6h}$) and AUC from 6 to 30 hours ($AUC_{ins.6–30h}$). Secondary PD endpoints included area under the GIR curve in the time intervals indicated above ($AUC_{GIR0–6h}$, $AUC_{GIR6–30h}$). Safety endpoints included adverse events (AEs), haematology, biochemistry, urine analysis, physical examinations, vital signs, ECGs, blood glucose levels, and local tolerability at the injection site.

2.3 | Assessments

Single-dose safety and local tolerability of the three treatments were assessed at each visit. Blood samples were collected (predose and approximately every 30 minutes during the 30-hour clamp period)

and used to determine study drug concentrations and related PK parameters.

No specific assays were commercially available to directly quantify MYL-1501D, US IG or EU IG; therefore, two complementary methods of assessment were used to evaluate therapeutic and metabolite quantification: a Mercodia enzyme-linked immunosorbent assay (ELISA) that cross-reacted with IG, M1 and human insulin was performed, but because these results may have been confounded by carryover effects of the baseline intravenous human insulin infusion, liquid chromatography with tandem mass spectrometry (LC–MS/MS) was also performed to specifically measure serum IG and the metabolites M1 and M2. Insulin glargine injected subcutaneously in humans is rapidly metabolized to M1 and M2, which retain the metabolic properties of IG and can be used to clarify the level of active drug available in plasma. Prior studies have found IG and M2 to be mostly undetectable; therefore, M1 concentrations were used for calculation of PK endpoints and for determination of bioequivalence.[13,14]

2.4 | Statistics

Statistical analyses were performed on the primary PK endpoints. To account for heteroscedasticity, $AUC_{ins.0-30h}$ and $C_{ins.max}$ were log-transformed and analysed using analysis of variance (ANOVA), with a mean value depending on insulin formulation and period as fixed effects and a random effect depending on subject. The IG formulation contrast was estimated together with the corresponding 90% confidence intervals (CIs). The estimates and upper and lower bounds of the 90% CIs were then exponentially transformed to find the estimated ratio of responses between the insulin formulations. If the exponentially transformed 90% CI for both $AUC_{ins.0-30h}$ and $C_{ins.max}$ fell within the limits of 0.80 and 1.25, bioequivalence was accepted. The LC–MS/MS analysis included only profiles with at least 12 quantifiable post-dose concentrations.

The same statistical approach with the same limits for the 90% and 95% CIs was assumed for the primary PD endpoints, $AUC_{GIR0-30h}$ and GIR_{max}. Smoothing of the GIR profiles was done with a locally weighted regression technique (LOESS, smoothing parameter 0.3). In a blinded data review, GIR profiles with very low response ($AUC_{GIR0-30h} \leq 50$ mg/kg; four profiles for MYL-1501D, five for US IG and five for EU IG) were excluded from the statistical model described above because a meaningful analysis of GIR parameters was not possible. A secondary sensitivity analysis included the log-transformed GIR profiles with very low response ($AUC_{GIR0-30h} \leq 50$ mg/kg). Another analysis that included the low profiles was based on the non-transformed data (assuming normality). A final sensitivity analysis including low responses used different cut-off values for data exclusion, ranging from 5 to 500 h·mg/kg/min (~0.5%–50% of the mean $AUC_{GIR0-30h}$). Secondary endpoints were compared using the same statistical approach as the primary endpoints. Bioequivalence criteria were not applied to the secondary PK/PD endpoints. Safety events were summarized using descriptive statistics.

3 | RESULTS

3.1 | Patient disposition and baseline characteristics

Of 141 patients screened, 114 were included in the safety population and randomized; 19 patients were assigned to each of the six treatment sequences. One patient was retrospectively excluded because of a C-peptide level that exceeded the inclusion criteria (0.58 pmol/mL). Accordingly, the PK and PD analysis set consisted of 113 patients. Two patients discontinued after treatment with EU IG at visit 2, both because of withdrawal of consent. Patient baseline characteristics are presented in Table 1. Forty patients had concomitant illnesses, including hypertension (n = 19), hypothyroidism (n = 12) and hyperlipidaemia (n = 5).

3.2 | Pharmacokinetic endpoints

Mean serum insulin PK (determined by ELISA) concentration profiles were similar between MYL-1501D, US IG and EU IG over the 30-hour analysis period (Figure 1A). On parametric statistical analysis of the log-transformed primary PK endpoints from the ELISA, geometric means of ratios between MYL-1501D, US IG and EU IG were close to 1 for both primary endpoints of $AUC_{ins.0-30h}$ and $C_{ins.max}$ (Table 2). The 90% CIs were within the defined range of 0.80 to 1.25.

The M1 analysis by LC–MS/MS demonstrated bioequivalence between MYL-1501D and both US IG and EU IG for the primary PK endpoints (Figure 1B; Table 3). Geometric means of ratios were close to 1, and 90% CIs were well within the acceptance range of 0.80 to 1.25. As many profiles showed <12 quantifiable post-dose concentrations and therefore were not included in the LC–MS/MS analysis, sensitivity analyses were performed that included profiles with lower minimum numbers of evaluable measurements up to all profiles. An

TABLE 1 Demographic and baseline characteristics of the safety population (N = 114)

Characteristics	Mean	SD
Age, years	39	9.5
Height, cm	179	8.5
Weight, kg	80	11
BMI, kg/m^2	25	2.5
C-peptide, nmol/L	0.03	0.07
HbA1c		
mmol/mol	59	8.5
%	7.54	0.78

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin.

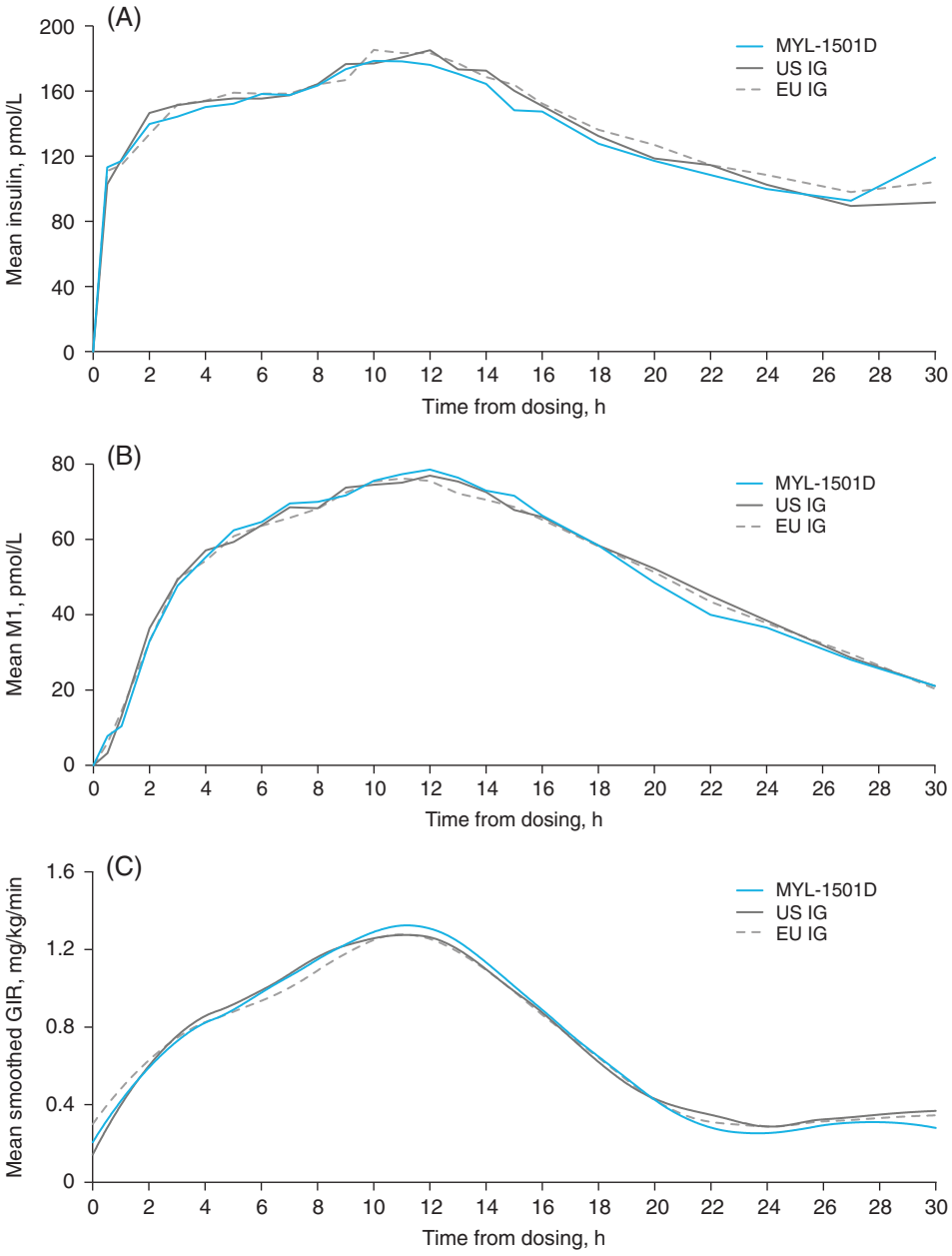

FIGURE 1 **A,** Mean smoothed serum insulin glargine (IG) profiles (enzyme-linked immunosorbent assay), **B,** mean plasma metabolite M1 profiles (liquid chromatography with tandem mass spectrometry), and **C,** mean smoothed glucose infusion rate (GIR) profiles of the three IG preparations in linear scale from injection at time 0 minutes to end of clamp procedure at 30 hours. EU IG, European reference insulin glargine; US IG, US reference insulin glargine

additional sensitivity analysis was based on M1 concentration with an extrapolated lower limit of quantitation of 0.1 ng/mL. All these analyses met the prespecified equivalence criteria (Table S1).

Although secondary PK endpoints were not planned to be used for proof of bioequivalence, analysis of $AUC_{ins.0-6h}$ and $AUC_{ins.6-30h}$ for M1 demonstrated that 90% CIs of the geometric means of treatment ratios were all within the range of 0.80 to 1.25 (Table 3).

3.3 | Pharmacodynamic endpoints

In the primary PD analysis, the GIR profiles were nearly superimposable for MYL-1501D, US IG and EU IG (Figure 1C). Geometric mean ratios between drugs were close to 1, and 90% and 95% CIs fell within the range of 0.80 to 1.25 (Table 4; 95% CIs presented in Table S2). The point estimates of the comparisons were close to 1 for $AUC_{GIR6-30h}$, indicating similar PD effects of the three drug formulations for this endpoint. Variability was high for $AUC_{GIR6-30h}$ and in particular for $AUC_{GIR0-6h}$ (an endpoint only covering the initial 6 hours post-dose, where the glucose-lowering effect of IG is usually low), so that the CIs were wide.

Sensitivity analyses were performed with different thresholds for low PD responses ranging from 5 to 500 h·mg/kg/min (ie, ~0.5%–50% of the mean $AUC_{GIR0-30h}$). For all tested thresholds >0, the 95% CIs were within the range of 0.80 to 1.25 and the mean ratios were close to unity (data not shown).

3.4 | Safety

Overall, 106 AEs were recorded in 66 of 114 patients (57.9%) during the study. Twenty-two patients (19.6%) experienced 38 AEs during

TABLE 2 Analysis of the primary pharmacokinetic endpoints (enzyme-linked immunosorbent assay)

	MYL-1501D (N = 110)	US IG (N = 107)[a]	EU IG (N = 112)
Geometric mean (90% CI)[b]			
$AUC_{ins.0-30h}$, pmol·h/L[c]	3013 (2717–3343)	3115 (2807–3456)	3167 (2856–3512)
$C_{ins.max}$, pmol/L	195 (178–213)	190 (174–208)	190 (174–208)
	MYL-1501D vs EU IG (N = 110)	**MYL-1501D vs US IG (N = 107)[a]**	**EU IG vs US IG (N = 107)[a]**
Geometric mean ratio (90% CI)[d]			
$AUC_{ins.0-30h}$, pmol·h/L	0.95 (0.89–1.02)	0.97 (0.90–1.04)	1.02 (0.95–1.09)
$C_{ins.max}$, pmol/L	1.02 (0.95–1.10)	1.02 (0.95–1.10)	1.00 (0.93–1.08)

Abbreviations: $AUC_{ins.0-30h}$, area under the serum insulin glargine concentration–time curve from 0 to 30 hours; CI, confidence interval; $C_{ins.max}$, maximum serum insulin glargine concentration; EU IG, European reference insulin glargine; US IG, US reference insulin glargine.
[a]For $C_{ins.max}$, N = 110.
[b]Parametric statistical analysis (analysis of variance) analysed using log transformation.
[c]Areas under the curve were calculated based on the linear trapezoidal rule and actual sampling time points.
[d]Geometric least squares mean ratios of treatments.

TABLE 3 Pharmacokinetic analysis of the M1 metabolite (liquid chromatography with tandem mass spectrometry)

	MYL-1501D (N = 87)	US IG (N = 87)	EU IG (N = 86)
Primary PK endpoints			
Geometric mean (90% CI)[a]			
$AUC_{ins.0-30h}$, pmol·h/L[b]	1328 (1226–1438)	1301 (1201–1409)	1310 (1209–1419)
$C_{ins.max}$, pmol/L	81.9 (76.7–87.4)	77.9 (72.9–83.1)	79.3 (74.2–84.6)
	MYL-1501D vs EU IG (N = 72)	**MYL-1501D vs US IG (N = 74)**	**EU IG vs US IG (N = 72)**
Geometric mean ratio (90% CI)[c]			
$AUC_{ins.0-30h}$, pmol·h/L[b]	1.01 (0.95–1.09)	1.02 (0.95–1.09)	1.01 (0.94–1.08)
$C_{ins.max}$, pmol/L	1.03 (0.97–1.10)	1.05 (0.99–1.12)	1.02 (0.96–1.08)
Secondary PK endpoints	**MYL-1501D (N = 85)[d]**	**US IG (N = 87)**	**EU IG (N = 84)[e]**
Geometric mean (90% CI)[a]			
$AUC_{ins.0-6h}$, pmol·h/L[b]	200 (178–226)	192 (171–217)	203 (180–229)
$AUC_{ins.6-30h}$, pmol·h/L[b]	1115 (1026–1210)	1086 (1000–1179)	1088 (1001–1181)
	MYL-1501D vs EU IG (N = 70)[f]	**MYL-1501D vs US IG (N = 73)[g]**	**EU IG vs US IG (N = 71)[f]**
Geometric mean ratio (90% CI)[c]			
$AUC_{ins.0-6h}$, pmol·h/L[b]	0.99 (0.86–1.12)	1.04 (0.91–1.18)	1.06 (0.93–1.20)
$AUC_{ins.6-30h}$, pmol·h/L[b]	1.02 (0.95–1.10)	1.03 (0.96–1.10)	1.00 (0.93–1.08)

Abbreviations: ANOVA, analysis of variance; $AUC_{ins.0-6h}$, area under the serum insulin glargine concentration–time curve from 0 to 6 hours; $AUC_{ins.0-30h}$, AUC from 0 to 30 hours; $AUC_{ins.6-30h}$, AUC from 6 to 30 hours; CI, confidence interval; $C_{ins.max}$, maximum serum insulin glargine concentration; EU IG, European reference insulin glargine; M1, metabolite M1; PK, pharmacokinetic; US IG, US reference insulin glargine.
[a]Parametric statistical analysis (ANOVA) analysed using log transformation.
[b]Areas under the curve were calculated based on the linear trapezoidal rule and actual sampling time points.
[c]Geometric least squares mean ratios of treatments.
[d]For $AUC_{ins.6-30h}$, N = 87.
[e]For $AUC_{ins.6-30h}$, N = 86.
[f]For $AUC_{ins.6-30h}$, N = 72.
[g]For $AUC_{ins.6-30h}$, N = 74.

TABLE 4 Analysis of pharmacodynamic endpoints

Parameter	MYL-1501D (N = 107)[a]	US IG (N = 106)[b]	EU IG (N = 107)[a]
Geometric mean (90% CI)			
Primary PD endpoints			
$AUC_{GIR0-30h}$, mg/kg[c]	956 (833–1099)	1022 (889–1174)	988 (860–1135)
GIR_{max}, mg/kg/min	1.38 (1.26–1.52)	1.40 (1.28–1.53)	1.38 (1.26–1.51)
Secondary PD endpoints	**MYL-1501D**	**US IG**	**EU IG**
	(N = 111)	**(N = 111)**	**(N = 113)**
$AUC_{GIR0-6h}$, mg/kg[c]	68.7 (46.4–102)	88.3 (59.7–131)	72.8 (49.4–107)
$AUC_{GIR6-30h}$, mg/kg[c]	556 (409–755)	522 (384–710)	567 (418–769)
$AUC_{GIR0-30h}$, mg/kg[c,d]	759 (597–965)[e]	772 (607–982)[e]	741 (584–941)[f]
	MYL-1501D	**MYL-1501D**	**EU IG**
	vs EU IG	**vs US IG**	**vs US IG**
	(N = 104)[g]	**(N = 103)**[h]	**(N = 104)**[h]
Geometric mean ratio (90% CI)[i]			
Primary PD endpoints			
$AUC_{GIR0-30h}$, mg/kg[c]	0.97 (0.85–1.11)	0.94 (0.82–1.07)	0.97 (0.85–1.11)
GIR_{max}, mg/kg/min	1.01 (0.92–1.10)	0.99 (0.91–1.08)	0.98 (0.90–1.07)
Secondary PD endpoints	**MYL-1501D**	**MYL-1501D**	**EU IG**
	vs EU IG	**vs US IG**	**vs US IG**
	(N = 111)	**(N = 111)**	**(N = 111)**
$AUC_{GIR0-6h}$, mg/kg[c]	0.94 (0.63–1.41)	0.78 (0.52–1.16)	0.82 (0.55–1.23)
$AUC_{GIR6-30h}$, mg/kg[c]	0.98 (0.72–1.34)	1.06 (0.78–1.46)	1.09 (0.79–1.48)
$AUC_{GIR0-30h}$, mg/kg[c,d]	1.02 (0.82–1.28)[e]	0.98 (0.78–1.23)[e]	0.96 (0.77–1.20)[e]

Abbreviations: $AUC_{GIR0-6h}$, area under the glucose infusion rate time curve from 0 to 6 hours; $AUC_{GIR0-30h}$, area under the glucose infusion rate time curve from 0 to 30 hours; $AUC_{GIR6-30h}$, area under the glucose infusion rate time curve from 6 to 30 hours; CI, confidence interval; EU IG, European reference insulin glargine; GIR_{max}, maximum glucose infusion rate; PD, pharmacodynamic; US IG, US reference insulin glargine.

[a]For GIR_{max}, N = 106.

[b]For GIR_{max}, N = 105.

[c]Areas under the curve were calculated based on the linear trapezoidal rule and actual sampling time points.

[d]This log-transformed endpoint was analysed using a linear mixed model with treatment and period as fixed factor and subject as a random factor.

[e]n = 111.

[f]n = 113.

[g]For GIR_{max}, N = 103.

[h]For GIR_{max}, N = 102.

[i]Geometric least squares mean ratios of treatments.

or after administration of MYL-1501D, 20 patients (17.9%) experienced 34 AEs with US IG, and 24 patients (21.1%) experienced 34 AEs with EU IG. The only AE that occurred in >5% of patients was headache, and there was one mild injection site reaction with US IG. There were no serious AEs, deaths or AEs leading to withdrawal. Twenty-one AEs were classified as treatment-related. Fifty-four episodes of hypoglycaemia (0 severe, 29 symptomatic, 25 asymptomatic) were recorded in 20 patients. Of these hypoglycaemic events, 19 occurred before first dosing, three occurred because of technical problems during the glucose clamp procedure (one with MYL-1501D and two with EU IG; blood glucose values of 3.3–3.4 mmol/L [60–61 mg/dL]), 31 occurred at various time points after the clamp period (nine with MYL-1501D, 16 with US IG and six with EU IG), and one occurred after the last treatment period. There were no abnormal laboratory results or changes that required reporting as an AE, nor any clinically significant changes in vital signs or physical examination. Abnormalities on ECGs were assessed and found not to be clinically significant.

4 | DISCUSSION

In the present study, PK and PD bioequivalence was shown among MYL-1501D, US IG and EU IG in patients with T1DM after receiving subcutaneous injections of 0.4 U/kg of the study drugs under automated euglycaemic clamp conditions. Bioequivalence was demonstrated for all primary PK and PD endpoints. Comparison of the three formulations by parametric analysis revealed that ratios of the geometric means for the primary PK parameters and for PD treatment ratios were very close to 1 (0.95–1.02 and 0.94–1.01, respectively),

demonstrating the similarity of MYL-1501D, US IG and EU IG. The limits of the 90% CIs of the ratios of $AUC_{ins.0-30h}$ and $C_{ins.max}$ ranged from 0.89 to 1.10, and $AUC_{GIR0-30h}$ and GIR_{max} geometric mean ratio 90% CIs ranged from 0.82 to 1.11, all within the prespecified range of 0.80 to 1.25, indicating bioequivalence. Even 95% CIs for the primary PD parameters fell within the bioequivalence margins. Pharmacokinetic findings were consistent between ELISA analyses of insulin levels and LC–MS/MS analyses of levels of the insulin metabolite M1. Although secondary PK characteristics were not used to determine bioequivalence, these findings support the conclusion of bioequivalence. The PK profiles of all three IG formulations (in particular, the profile of the major metabolically active metabolite, M1) are in line with data from a previous study in which M1 was analysed by LC–MS/MS.[13] Interestingly, both the current data and this previous study show M1 concentrations that are considerably above the lower limit of quantification at 30 hours, while PD activity returns to near-baseline level at ~24 hours. While this phenomenon has previously been observed, it has not been extensively discussed. However, during review of the present manuscript, one of the reviewers suggested this may be attributable to the detection of (partially) inactive or degraded forms of the molecule, which is certainly a potential explanation for this phenomenon.

As part of the secondary PD findings, some patients presented very low profiles with $AUC_{GIR0-30h} \leq 50$ mg/kg. When these low responders were included in the analysis, equivalence was not formally shown. Guidelines from the EMA for recombinant human insulin and insulin analogues state that while PD results should reasonably support PK results, all GIR-related variables may be defined as secondary endpoints if close similarity in physicochemical and functional characteristics are shown for the biosimilar and reference insulin.[11] While these secondary variables were not used for the determination of bioequivalence, the mean ratios for all presented analyses were close to 1, low responses were not always seen in the same patients and occurred inconsistently (usually just one in three clamps), and were equally distributed among treatments. These differences are best explained by intra-individual variability of study patients and do not suggest that the PD results falling slightly outside of the prespecified 0.80 to 1.25 margins are attributable to true differences in PD behaviour. The additional sensitivity analysis including low responders using different data exclusion thresholds showed 95% CIs within expected ranges, implying that patients with low responses did not introduce bias. Overall, these PD results reasonably support the PK results.

This study used a classical crossover design for a bioequivalence trial such that each patient acted as their own control, reducing variability in each of the measured variables, and also compared the two reference products with each other. The dose of 0.4 U/kg was chosen to provide a robust dose–response in patients with T1DM and is within the dose range recommended for clinical glucose clamp trials. The euglycaemic clamp technique is widely used to evaluate insulin activity and is recommended by the EMA for the demonstration of biosimilarity between insulins in clinical pharmacology studies.[11] Patients with T1DM were chosen for this study not only to provide data relevant to real-world populations who use IG but also because these patients lack endogenous insulin, allowing the accurate determination of each insulin's time–action profile without the confounder of competing endogenous insulin.[15] This is in contrast with some biosimilar IG programmes that focus on euglycaemic clamp studies in healthy people without T1DM.[14,16,17] There is agreement among clamp experts that PD outcomes such as overall activity, particularly toward the end of clamp procedures, may be affected by endogenous insulin secretion during glucose clamp tests in both healthy people and in people with T2DM, which may lead to an increase in GIR.[18,19] Variables such as $AUC_{GIR6-30h}$ and total AUC can therefore only be reliably determined in people with T1DM, which may explain why other IG biosimilar programmes included clamp tests in this population.[18-20] The present study in people with T1DM allowed the comparison of overall activity across the three study IG formulations, establishing this aspect of the proof of bioequivalence. Furthermore, the observed PD profiles of all three IG formulations (showing a return to near-baseline levels at ~24 hours and a subtle peak effect at ~10–12 hours) are in line with previously published glucose clamp profiles of this insulin analogue.[13] The clinical properties of the three IG formulations should therefore be similar to those already published for IG, with less hypoglycaemia (in particular, nocturnal hypoglycaemia) compared with NPH insulin,[21] but slightly more hypoglycaemia compared with second-generation basal insulin analogues (eg, insulin glargine U300 [in patients with T2DM] or insulin degludec), which show less peak effect than IG in glucose clamp studies.[22]

MYL-1501D, US IG and EU IG were generally well tolerated, and no significant safety issues emerged. Twenty-one AEs were classified as treatment-related and the most prevalent AE was headache, which has been commonly reported in numerous other glucose clamp studies with IG and other insulins.[23,24] Because of the use of the glucose clamp technique, hypoglycaemia, which is the most common adverse reaction with insulins, was avoided for 30 hours post dose, with the exception of three mild events resulting from technical difficulties. The other hypoglycaemic events in this study occurred before or > 30 hours after injection and were therefore considered to be a side effect of the patients' usual insulin treatment. Moreover, in this study, there were no noticeable differences in the safety profiles among study drug formulations with regard to type, frequency and severity of AEs. Phase 3 studies have demonstrated the efficacy and safety of MYL-1501D in comparison with reference IG in patients with T1DM (ClinicalTrials.gov identifier, NCT02227862) and T2DM (ClinicalTrials.gov identifier, NCT02227875).[25,26]

In conclusion, this study demonstrated bioequivalence among the proposed biosimilar MYL-1501D, US IG and EU IG when administered as single subcutaneous injections of 0.4 U/kg as measured by the primary PK and PD endpoints. Overall, all three study drugs were well tolerated, and no significant safety issues arose. Results from this study provide further support that the proposed IG biosimilar MYL-1501D may be appropriate for clinical use in patients with T1DM, as indicated by phase 3 studies.[25,27]

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PRIOR PRESENTATION

These data were previously presented in part at the 77th Scientific Sessions of the American Diabetes Association; June 9–13, 2017; San Diego, California.

CONFLICT OF INTEREST

T.H. is a member of advisory panels for Novo Nordisk and Mylan Inc., has received speaker honoraria and travel grants from Eli Lilly and Novo Nordisk, and his institution has received research funds from Adocia, Boehringer Ingelheim, Biocon Ltd, Dance Pharmaceuticals, Eli Lilly, Gan & Lee Pharmaceuticals, Johnson & Johnson, Mars, MedImmune, Mylan Inc., Nordic Bioscience, Novo Nordisk, Pfizer, Poxel, Saniona, Sanofi, Wockhardt, and Zealand Pharma. C.D., A.B. and P.A. are paid employees of Mylan Inc. and may hold stock in the company.

AUTHOR CONTRIBUTIONS

T.H. contributed to the design of the study, the acquisition, analysis and interpretation of data, and critically revising the manuscript for important intellectual content. C.D. contributed to the acquisition and analysis of data. A.B. contributed to the conception of the study, the design of the study, the analysis and interpretation of data, and critically revising the manuscript for important intellectual content. P.A. contributed to the interpretation of data and critically revising the manuscript for important intellectual content. All authors approved the manuscript for publication.

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SUPPORTING INFORMATION

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

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