



Immunogenicity, Efficacy, and Safety of Biosimilar Insulin Aspart (MYL-1601D) Compared with Originator Insulin Aspart (Novolog®) in Patients with Type 1 Diabetes After 24 Weeks: A Randomized Open-Label Study

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Abstract

Background MYL-1601D is a proposed biosimilar of originator insulin aspart, Novolog®/NovoRapid® (Ref-InsAsp-US/Ref-InsAsp-EU).

Objective This study assessed the immunogenicity, efficacy, and safety of MYL-1601D with Ref-InsAsp-US in patients with type 1 diabetes mellitus (T1D).

Methods This was a 24-week, open-label, randomized, phase III study. Patients were randomized 1:1 to mealtime MYL-1601D or Ref-InsAsp-US in combination with insulin glargine (Lantus SoloSTAR®) once daily. The treatment-emergent antibody response (TEAR) rate (defined as patients who were anti-insulin antibody [AIA] negative at baseline and became positive at any timepoint post-baseline or patients who were AIA positive at baseline and demonstrated a 4-fold increase in titer values at any timepoint post-baseline) was the primary endpoint. The study also compared the change from baseline in glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), prandial, basal, and total daily insulin, 7-point self-monitored blood glucose (SMBG) profiles, immunogenicity, and adverse events (AEs) including hypoglycemia.

Results In total, 478 patients were included in the intent-to-treat analysis (MYL-1601D: 238; Ref-InsAsp-US: 240) set. The 90% confidence interval (CI) for the primary endpoint was within the pre-defined equivalence margin of $\pm 11.7\%$ and the treatment differences (SE) in TEAR responders between the treatment groups was -2.86 (4.16) with 90% CI -9.71 to 3.99. The mean (SD) changes from baseline for HbA1c, FPG, and insulin dosages were similar in both groups at week 24. The safety profiles including hypoglycemia, immune-related events, AEs, and other reported variables were similar between the treatment groups at week 24.

Conclusions MYL-1601D demonstrated similar immunogenicity, efficacy, and safety profiles to Ref-InsAsp-US in patients with T1D over 24 weeks.

Clinical Trial Registration [ClinicalTrials.gov: NCT03760068](https://clinicaltrials.gov/ct2/show/study/NCT03760068).

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Key Points

A rapid-acting version of human insulin (MYL-1601D) of recombinant DNA origin was developed as a biosimilar to the already approved insulin aspart formulations (NovoLog® in the US). It is indicated to improve glycemic control in adults and pediatric patients with diabetes mellitus.

This phase III study in patients with type 1 diabetes confirmed that MYL-1601D had similar immunogenicity, efficacy, and safety profiles to the reference product (NovoLog®).

MYL-1601D biosimilar product provides an alternative treatment option for insulin aspart.

1 Introduction

Maintaining good glycemic control is critical to prevent or delay microvascular and macrovascular complications in patients with type 1 (T1D) and type 2 diabetes mellitus (T2D) [1–3]. The principal treatment of T1D is the initiation of insulin therapy, diet control, and careful monitoring of blood glucose levels. The American Diabetes Association (ADA) standard-of-care recommends a basal-bolus insulin regimen with one or two daily injections of long/intermediate-acting insulin covering basal insulin requirements in combination with three daily injections of short/rapid-acting insulin to cover meal-related insulin requirements, which yields the best glycemic control in diabetes. Clear targets for plasma glucose levels have been recommended by the ADA for basal-bolus insulin regimens [4].

Insulin aspart is a rapid-acting insulin analog, available in the United States as Novolog[®] (Ref-InsAsp-US) and in the European Union (EU) as NovoRapid[®] (Ref-InsAsp-EU). Several randomized controlled trials (RCTs) have demonstrated the safety and efficacy of insulin aspart in diverse patient populations with T1D and T2D [5].

A biosimilar drug is a biological product that is highly similar to a licensed biological product with no clinically meaningful differences in terms of safety, purity, or potency [6, 7]. The introduction of biosimilar insulins provides an option to reduce diabetes treatment costs, improve accessibility to new insulin treatment options, and expand the number of insulin brands available for individuals with diabetes [8].

MYL-1601D (insulin aspart solution 100 U/mL) is being developed as a biosimilar product to originator insulin aspart. MYL-1601D is produced by recombinant DNA technology utilizing *Pichia pastoris* (yeast) in accordance with relevant United States and EU guidelines. The qualitative and quantitative composition of MYL-1601D has been identical to that of Ref-InsAsp-US and Ref-InsAsp-EU in physicochemical analyses and nonclinical studies with no clinically meaningful differences observed [9]. A euglycemic clamp study demonstrated pharmacokinetic (PK) and pharmacodynamic (PD) similarity of MYL-1601D versus both Ref-InsAsp-US and Ref-InsAsp-EU in healthy volunteers [10].

This article reports the findings of the phase III study comparing the immunogenicity, efficacy, and safety of treatment with MYL-1601D biosimilar and reference product Ref-InsAsp-US in patients with T1D at 24 weeks with a primary focus on immunogenicity and its potential clinical impact on safety and efficacy.

2 Methods

2.1 Study Design and Participants

This was a 24-week, multicenter, open-label, randomized, parallel-group, phase III study in patients with T1D comparing immunogenicity, safety, and efficacy of MYL-1601D with Ref-InsAsp-US. It was conducted in 149 centers in the United States. The study comprised a 4-week run-in period, a 24-week treatment period, and a 4-week follow-up period. Patients were randomized in a 1:1 ratio to receive either MYL-1601D (100 U/mL) or reference Ref-InsAsp-US (100 U/mL) once daily. A follow-up visit, via a telephone call, was scheduled 4 weeks after the last dose of MYL-1601D or Ref-InsAsp-US.

For the study, patients aged 18–65 years, with a body mass index of 18.5–35.0 kg/m², and established diagnosis of T1D were included. All patients were on insulin treatment (stable dose of once-daily basal Lantus or Toujeo injection and multiple daily bolus Ref-InsAsp-US or Humalog injections) for a minimum of 3 months before screening. At screening, patients had a glycated hemoglobin (HbA1c) concentration of 6.5–10.0% and a hemoglobin of ≥ 10.0 g/dL. Key exclusion criteria included patients with a history of clinically significant infections and medical conditions, autoimmune disorders, history of hematological disorders, insulin pump usage in the last 3 months before screening, any non-insulin antidiabetic therapies, clinically significant abnormal laboratory data, secondary diabetic complications of moderate insulin, moderate insulin resistance, and patients who planned to receive elective surgery during the study period.

The clinical study protocol and other essential clinical documents were reviewed and approved by an institutional review board/ethics committee at each clinical site. This study was conducted in accordance with legal and regulatory requirements, International Ethical Guidelines for Biomedical Research Involving Human Patients [11], International Council for Harmonisation Good Clinical Practice (ICH-GCP) [12], and the Declaration of Helsinki [13]. Written informed consent was obtained from all patients prior to study inclusion. The study was registered at ClinicalTrials.gov (NCT03760068).

2.2 Study Treatments

After a 3-week screening period, during the run-in period, all patients received subcutaneous injection of FlexPen[®] Ref-InsAsp-US at a concentration of 100 U/mL (Batch numbers: HZF7372; HZFA196; JZFC499; JZFC879; manufactured by Novo-Nordisk) at mealtime until randomization. In addition, all patients were shifted from their current basal

insulin to Lantus SoloSTAR[®] (insulin glargine injection, 100 U/mL, manufactured by Sanofi-Aventis) once daily at the start of the run-in period and continued till study completion. The doses of Ref-InsAsp-US and Lantus were titrated during the run-in period to ensure diabetes control. During the treatment period, all patients received one of the following treatments: MYL-1601D (Batch number: BM18002196) manufactured by Biocon from a manufacturing process or Ref-InsAsp-US taken at mealtime in a prefilled disposable pen with a 3-mL cartridge. Frequent adjustments of insulin dose were discouraged. Study treatment dose and titration instructions were given to the patient at the time of medication dispense, and all subsequent study visits were as per the recommended ADA 2019 standard-of-care specifics (the targets were prandial or post prandial). The 7-point self-monitoring of blood glucose (SMBG) diary was assessed, reviewed, and discussed at each visit to ensure the effectiveness and safety of glycemic control.

2.3 Primary Endpoint

The primary endpoint was treatment-emergent antibody response (TEAR) rate during the 24-week treatment period. In this study, TEAR was defined as patients who were anti-insulin antibody (AIA) negative at baseline and became positive at any timepoint post-baseline (treatment-induced AIA) or patients who were AIA positive at baseline and had 4-fold increase in titer value at any time post-baseline (treatment-boosted AIA). The criteria of a 4-fold increase was considered a scientifically reasonable margin to define treatment-boosted AIA [14, 15]. The TEAR rate was not assessed in isolation but was part of the totality of evidence including changes in HbA1c, fasting plasma glucose (FPG), insulin dose, neutralizing antibodies (NAb), and injection-site reaction (ISR) to determine if any observed changes in TEAR rate were clinically meaningful.

2.4 Efficacy Endpoints

Efficacy endpoints were assessed as secondary endpoints and included change from baseline to week 24 in HbA1c, FPG, prandial, basal, and total daily insulin dose per unit body weight (U/kg), and 7-point SMBG profile.

2.5 Safety Assessments

Safety endpoints included incidence of positive antibody response and NAb, impact of AIA on PD parameters, such as FPG, HbA1c, and insulin dose, change in hypoglycemia rate (30-day adjusted), incidence of hypoglycemic events,

incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs), ISRs, systemic reactions, hypersensitivity, and immune-mediated AEs, and device-related safety assessments. All subjects received a diary and blood glucose monitoring device (glucometer) at week – 4 to monitor blood glucose at home and to record the 7-point SMBGs. The incidence of hypoglycemic episodes was summarized by category; severe hypoglycemia (an event requiring the help of another person to actively administer carbohydrate, glucagon, or different resuscitative actions), documented symptomatic hypoglycemia (an event of hypoglycemia accompanied by a measured plasma glucose concentration ≤ 70 mg/dL [3.9 mmol/L]), asymptomatic hypoglycemia (an event of hypoglycemia not accompanied by a plasma glucose concentration ≤ 70 mg/dL [3.9 mmol/L]), probable symptomatic hypoglycemia (hypoglycemia with no glucose level activity that resolved with food intake, body covering endocrine, or endogenous glucose), relative hypoglycemia (an event of hypoglycemia accompanied by a plasma glucose concentration > 70 mg/dL [3.9 mmol/L]), and nocturnal hypoglycemia (hypoglycemia that happens from the time the patient goes to bed at nighttime until the time he or she wakes up) were considered as serious adverse events [16, 17].

2.6 Immunogenicity Assessments

Pre-dose serum samples for immunogenicity assessments were collected at baseline and at pre-specified time points of 2, 4, 8, 12, 16, 20, and 24 weeks after randomization. A conventional radioimmunoassay was used to detect the presence of AIAs in a tiered approach (screening, confirmation, and characterization) [14, 18] using 125I-MYL-1601D as tracer. All confirmed AIA positive samples were evaluated in the characterization tier which included titer determination and evaluation of insulin cross-reactivity using excess human insulin in the confirmatory tier. During method validation, the screening cut point factor (1.27), confirmatory cut point (47.2%), and titer cut point factor (1.66) were determined in healthy human sera using the statistical methods consistent with robust procedures recommended by Shankar et al. [14] and Devanarayan et al. [19]. The sensitivity of the screening and confirmatory assays was 10.36 ng/mL and 16.03 ng/mL at the 95% consistency level, respectively, and both assays exhibited precision of $< 20\%$ CV at the low positive control level. The titration assay precision yielded a minimum significant ratio of 2 [20], sufficient to support the significance of a 4-fold increase in titer for treatment-boosted ADA. Confirmed AIA positive samples were also tested for the presence of NAb using a separate cell-based assay in which the inhibition of insulin receptor phosphorylation in a transfected Chinese hamster ovary cell line overexpressing insulin receptor was measured.

2.7 Statistical Analysis

The sample size estimation was planned with approximately 500 patients based on the primary objective. The 80% power would be achieved with 250 patients per treatment group to demonstrate that the 90% confidence interval (CI) of treatment difference (MYL-1601D minus Ref-InsAsp-US) is within the margin.

The equivalence margin was provided by the regulatory agency with fixed sample size ($n = 500$) based on publications by Chow et al. [21, 22]. The margin was determined by the TEAR rate of the reference treatment group. However, due to the nature of binomial distribution, the margin is largest when the reference TEAR rate is 50%, where the variance is largest, and margins decrease as reference rates increase or decrease away from 50%. The final TEAR rate for primary analysis in the Ref-InsAsp-US group was 27.8% and therefore the final margin of $\pm 11.7\%$ was derived based on the reference group rate and 500 planned sample size. The margins for sensitivity analyses were calculated according to different TEAR rates in the reference treatment group (Fig. S1, see electronic supplementary material [ESM]).

Missing data were imputed using multiple imputation methods for primary and sensitivity analysis for TEAR rate associated with AIA and secondary variables. A different imputation process was used for each of the two TEAR criteria: (i) logistic regression multiple imputations assuming missing not at random for baseline AIA-negative patients, post-baseline binary response (positive or negative); (ii) for baseline positive patients, missing titer values (continuous values) were imputed with the same treatment group non-missing patients using the pattern mixture model with the complete-case missing values method [23].

All statistical procedures were performed using Statistical Analysis Software (SAS[®]) 9.3 or higher (SAS Institute, Cary, NC, USA). The 90% CI of treatment difference in TEAR rate was established using the Wald confidence limit method. The TEAR rate with AIA of MYL-1601D was established equivalent to Ref-InsAsp-US if the 90% CI of the treatment difference was within the margin of -11.7% to 11.7% . For secondary and safety continuous variables, a mixed model repeated measures (MMRM) model was used; for HbA1c and FPG, actual values and changes from baseline at each timepoint were captured and summarized by the treatment group. For HbA1c, treatment difference and 95% CIs were also displayed along with p -values. Sensitivity analyses were performed to evaluate the impact of missing data on primary analysis results.

Safety data were summarized using descriptive statistics. Adverse events (serious and non-serious) were graded in accordance with the NCI-CTCAE scale [24]. The hypoglycemic event rate per subject per 30 days was analyzed

using MMRM. For antibody continuous variables, Fisher's exact test or Chi-squared test was used for categorical data analyses.

3 Results

3.1 Study Participants

A total of 528 patients were randomized and received either one dose of MYL-1601D ($n = 263$) or Ref-InsAsp-US ($n = 265$); of these, 441 (224 [94.1%] vs 217 [90.4%]) completed the study. A total of 50 patients at one clinical site were excluded before database lock and data analysis from intent-to-treat (ITT), per-protocol (PP) and safety analysis sets due to GCP violations. Hence, the ITT sets included a total of 478 patients (MYL-1601D, 238; Ref-InsAsp-US, 240). Twenty (7.6%) patients in the MYL-1601D group and 23 (8.7%) patients in the Ref-InsAsp-US group discontinued treatment, with the most frequent reasons being requests by patient (MYL-1601D, 13 [4.9%]; Ref-InsAsp-US, 8 [3.0%]) and lost to follow up (3 [1.1%] vs 9 [3.4%]).

Patients had a mean age of 44.5 years in MYL-1601D and 44.2 years in Ref-InsAsp-US and had a mean duration of diabetes of 21.8 years. Overall, the demographic and baseline characteristics were similar between the two treatment groups (Table 1).

3.2 Primary Outcome

Overall, the number of patients (MYL-1601D, 59 [24.9%]; Ref-InsAsp-US, 67 [27.8%]) who were TEAR positive (response rate) was comparable between the treatment groups; the treatment difference (SE) in TEAR responders between the treatment groups was -2.86 (4.16) (90% CI, -9.71 to 3.99). Based on the observed TEAR rate, the equivalence margin for the primary efficacy analysis was $\pm 11.7\%$, thus meeting the criteria for equivalence between MYL-1601D and Ref-InsAsp-US. The results of the sensitivity analyses without imputation in the ITT set and the PP analysis set were also similar to the primary analysis (Fig. S1, see ESM).

3.3 Immunogenicity Outcomes

A summary of AIA responses occurring in the study is presented in Table 2. Post-baseline, 24 (10.1%) patients in the MYL-1601D group and 35 (14.6%) patients in the Ref-InsAsp-US group reported a newly positive AIA (treatment-induced AIA), the differences between groups were not statistically significant ($p = 0.13$). A proportion of 28 (11.8%) patients in the MYL-1601D group and 22 (9.2%) patients in the Ref-InsAsp-US group reported a 4-fold increase in

Table 1 Participant demographics and baseline characteristics

Variable	MYL-1601D (N = 238)	Ref-InsAsp-US (N = 240)
Age, mean (SD), years	44.5 (13.33)	44.2 (13.91)
Gender, n (%)		
Male	129 (54.2)	132 (55.0)
Female	109 (45.8)	108 (45.0)
Race, n (%)		
White	210 (88.2)	210 (87.5)
Black or African American	15 (6.3)	11 (4.6)
Native Hawaiian or Other Pacific Islander	3 (1.3)	2 (0.8)
Asian	6 (2.5)	9 (3.8)
American Indian or Alaska Native	1 (0.4)	0
Other	3 (1.3)	8 (3.3)
Ethnicity, n (%)		
Hispanic or Latino	57 (23.9)	45 (18.8)
Not Hispanic or Latino	181 (76.1)	195 (81.3)
BMI, mean (SD), kg/m ²	27.56 (4.221)	27.00 (4.191)
Duration of diabetes, mean (SD), years	22.3 (13.71)	21.3 (12.91)
Time of basal dose, n (%)		
Morning	84 (35.3)	91 (37.9)
Evening	154 (64.7)	149 (62.1)
FPG, mean (SD), mmol/L	N = 237 8.944 (3.9321)	N = 237 9.218 (3.8593)
Baseline HbA1c, mean (SD), %	N = 238 7.85 (0.868)	N = 240 7.81 (0.784)
Insulin use prior to screening, n (%)		
Yes	238 (100)	238 (100)
No	0	0

BMI body mass index; FPG fasting plasma glucose, HbA1c glycated hemoglobin, ITT Intent-to-treat, N number of patients in the population, n number of patients with data, SD standard deviation

AIA titer (treatment-boosted AIA), the differences between groups were not statistically significant ($p = 0.35$). Overall, the incidence of AIA response demonstrated similar profiles between the two treatment groups.

The incidence of positive insulin cross-reactive response among patients with AIA-positive status was similar between the two treatment groups. The profiles of HbA1c and total insulin dose were also comparable between TEAR positives and TEAR negatives. The hypoglycemic rate was also shown to be similar between the two treatment groups for both TEAR positive and negative groups. Both the positive and negative groups experienced low ISRs and hypersensitivity events.

The number of patients with positive NAb samples from the overall AIA-positive samples was low and comparable between the two treatment groups. At baseline, before MYL-1601D treatment, a proportion of patients (MYL-1601D, 189 [79.4%]; Ref-InsAsp-US, 170 [70.8%]) reported AIA positivity. Of these, 15 (6.3%) and 17 (7.1%) patients were NAb positive at baseline in the MYL-1601D and Ref-InsAsp-US

groups, respectively (Table 3). In general, patients who were TEAR positive at any post-baseline visit had a very low positive NAb incidence rate that was comparable between MYL-1601D and Ref-InsAsp-US groups. Of the patients with TEAR-positive status, 4 (7.7%) in the MYL-1601D group and 4 (7.0%) in the Ref-InsAsp-US group were NAb positive and 48 (92.3%) in the MYL-1601D group and 53 (93%) in the Ref-InsAsp-US group were NAb negative.

3.4 Secondary Efficacy Outcomes

In both groups, HbA1c remained relatively stable throughout the treatment period. The mean (SD) change from baseline in HbA1c at week 24 was 0.10% (0.74%) in the MYL-1601D group and 0.04% (0.72%) in the Ref-InsAsp-US group with treatment difference (MYL-1601D – Ref-InsAsp-US) of 0.07 (95% CI – 0.06 to 0.20) (Table 3 and Fig. 1A). The mean (SD) change from baseline fasting plasma glucose at week 24 was 0.40 (5.82) mmol/L in the MYL-1601D group and 0.60 (5.16) mmol/L in the Ref-InsAsp-US group with

Table 2 Summary of anti-insulin antibody response at week 24

Characteristics, <i>n</i> (%)	MYL-1601D (<i>N</i> = 238)	Ref-InsAsp-US (<i>N</i> = 240)	<i>p</i> value ^a
AIA negative at baseline	48 (20.2)	69 (28.8)	NA
Newly positive post-baseline AIA (treatment-induced AIA)	24 (10.1)	35 (14.6)	0.13
AIA positive at baseline	189 (79.4)	170 (70.8)	NA
With 4-fold increase in titer (treatment-boosted AIA)	28 (11.8)	22 (9.2)	0.35
With at least 1 positive AIA at any treatment visit	210 (88.2)	202 (84.2)	0.19
With TEAR	52 (21.8)	57 (23.8)	0.62
Without TEAR	163 (68.5)	157 (65.4)	0.47
Unconfirmed TEAR status ^b	23 (9.7)	26 (10.8)	0.67
Patients who met TEAR, HbA1c, and dose criteria at any visit	14 (5.9)	12 (5.0)	0.67
Incidence of AIA and NAb ^c			
AIA positive at baseline	189 (79.4)	170 (70.8)	0.73
Nab positive	15 (6.3)	17 (7.1)	
AIA positive post-baseline	174 (73.1)	166 (69.2)	
Nab positive	11 (4.6)	15 (6.3)	0.54

Percentages were based on *N*

For the TEAR analysis, an imputation of missing values was performed, and no patient was excluded from the primary analysis

AIA anti-insulin antibody, HbA1c glycated hemoglobin, *N* number of patients in population, *n* number of patients with data, NA not applicable, NAb neutralizing antibodies, TEAR treatment emergent antibody response

^a*p* value was based on Chi-Square test if 80% of the cells had an expected frequency ≥ 5 , Fisher's exact test was used otherwise

^bPatients with at least 1 missing scheduled visit data and TEAR positive status could not be determined

^cPatients with missing sample were not displayed

Table 3 Summary of secondary efficacy outcomes at week 24

Treatment variable	Baseline Mean (SD) [<i>n</i>]	Week 24 Mean (SD) [<i>n</i>]	Change from baseline to week 24 Mean (SD) [<i>n</i>]	Change from baseline to week 24 LS mean (SE) [<i>n</i>]	MYL-1601D-Ref- InsAsp-US treatment difference (95% CI)
<i>HbA1C</i> , %					
MYL-1601D (<i>N</i> = 238)	7.85 (0.86) [229]	7.93 (0.94) [226]	0.10 (0.74) [217]	0.11 (0.05) [217]	0.07 (– 0.06 to 0.20)
Ref-InsAsp-US (<i>N</i> = 240)	7.80 (0.77) [235]	7.82 (0.93) [216]	0.04 (0.72) [211]	0.04 (0.05) [211]	
<i>FPG</i> , mmol/L					
MYL-1601D (<i>N</i> = 238)	9.02 (4.03) [230]	9.33 (4.78) [220]	0.40 (5.82) [214]	0.36 (0.31) [214]	– 0.36 (– 1.22 to 0.50)
Ref-InsAsp-US (<i>N</i> = 240)	9.28 (3.98) [236]	9.79 (4.36) [211]	0.60 (5.16) [208]	0.72 (0.31) [208]	
<i>Daily prandial insulin dose</i> , U/kg/day					
MYL-1601D (<i>N</i> = 238)	0.38 (0.21) [221]	0.39 (0.21) [200]	0.0079 (0.1461) [193]	0.0102 (0.0082) [193]	0.01 (– 0.01 to 0.03)
Ref-InsAsp-US (<i>N</i> = 240)	0.36 (0.18) [220]	0.37 (0.19) [199]	– 0.0008 (0.0973) [190]	– 0.0001 (0.0082) [190]	
<i>Daily basal insulin dose</i> , U/kg/day					
MYL-1601D (<i>N</i> = 238)	0.37 (0.14) [236]	0.38 (0.15) [221]	0.0053 (0.0646) [219]	0.0078 (0.0039) [219]	0.0049 (– 0.0058 to 0.0156)
Ref-InsAsp-US (<i>N</i> = 240)	0.37 (0.16) [237]	0.38 (0.16) [211]	0.0001 (0.0515) [210]	0.0029 (0.0039) [210]	
<i>Daily total insulin dose</i> , U/kg/day					
MYL-1601D (<i>N</i> = 238)	0.75 (0.29) [219]	0.77 (0.29) [198]	0.02 (0.16) [189]	0.0210 (0.0093) [189]	0.0172 (– 0.0082 to 0.0426)
Ref-InsAsp-US (<i>N</i> = 240)	0.74 (0.27) [218]	0.74 (0.27) [195]	0.0024 (0.1065) [185]	0.0038 (0.0094) [185]	

MYL-1601D Mylan Insulin Aspart; in addition to the originator product name NovoLog, the product code name, Ref InsAsp-US is used throughout the document

FPG fasting plasma glucose, HbA1c glycated hemoglobin, SD standard deviation, SE standard error

treatment difference of -0.36 (95% CI -1.22 to 0.50) (Table 3 and Fig. 1B). The treatment difference at any visit was not statistically significant. There were no notable trends between treatment groups at baseline and week 24 in seven-point SMBG profile (Table S1, see ESM).

The mean (SD) change from baseline in daily mealtime insulin dose at week 24 was 0.0079 (0.1460) U/kg in the MYL-1601D group and -0.0008 (0.0973) U/kg in the Ref-InsAsp-US group with treatment difference of 0.01 (95% CI -0.01 to 0.03) (Table 3 and Fig. 1C). The mean (SD) change from baseline total daily insulin dose at week 24 was 0.02 (0.16) U/kg in the MYL-1601D group; 0.0024 (0.1064) U/kg in the Ref-InsAsp-US group with treatment difference of 0.0172 (95% CI -0.0082 to 0.0426) (Table 3 and Fig. 1D).

3.5 Safety Outcomes

MYL-1601D and Ref-InsAsp-US were found to have similar safety profiles (Table S2, see ESM). A total of 256 TEAEs were reported in 108 (45.4%) patients in the MYL-1601D group and 231 TEAEs in 103 (42.9%) patients in the Ref-InsAsp-US group. The most frequent TEAEs were nasopharyngitis, hypoglycemia, and upper respiratory tract infection reported by 14 (5.9%), 17 (7.1%), and 11 (4.6%) patients in the MYL-1601D group, and 15 (6.3%), 10 (4.2%), and 14 (5.8%) patients in the Ref-InsAsp-US group, respectively. A low number of patients with hypersensitivity and immune-mediated TEAEs were reported in both treatment groups (Table S2, see ESM).

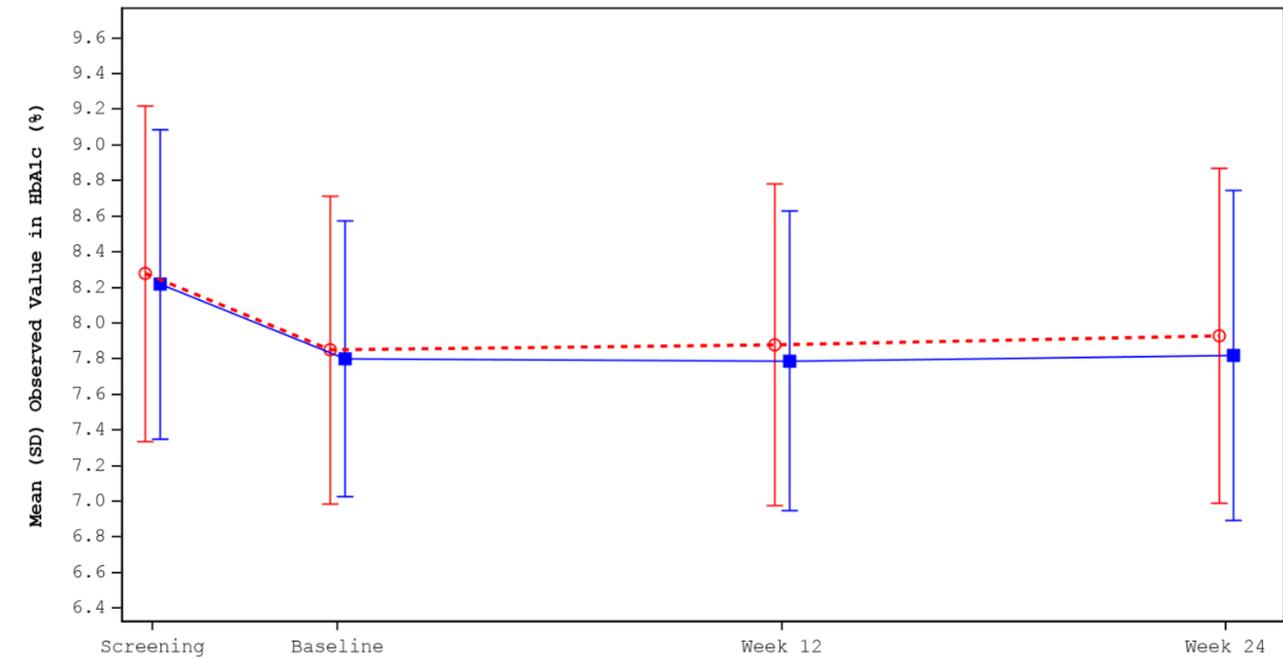
The SAE reports were numerically higher in the MYL-1601D group but not statistically significant; 22 (9.2%) patients in the MYL-1601D group reported 27 SAEs and 15 (6.3%) patients reported 23 SAEs in the Ref-InsAsp-US group. The most frequently reported SAE in both the treatment groups was hypoglycemia (15 [6.3%] vs 10 [4.2%]). All SAEs, except hypoglycemia, were reported only once. One (0.4%) patient in the Ref-InsAsp-US group died due to homicide during the study (Table S4, see ESM).

There was a numerical difference in incidence of hypoglycemia TEAEs between the treatment groups (17 [7.1%] patients in the MYL-1601D group vs 10 [4.2%] patients in the Ref-InsAsp-US group). In addition to the reporting of the AE by the investigator, patients were instructed to record any hypoglycemic event based on symptoms or actual glucometer measurement in the study diary. The overall incidence of hypoglycemic events per the study diary was similar for both treatment groups (218 [91.6%] patients in the MYL-1601D group and 222 [92.5%] patients in the Ref-InsAsp-US group) at any visit. Of these, 15 (6.3%) patients in each treatment arm had severe hypoglycemia at any visit. The mean hypoglycemic event rates (episodes per 30 days adjusted) were comparable between the treatment groups up

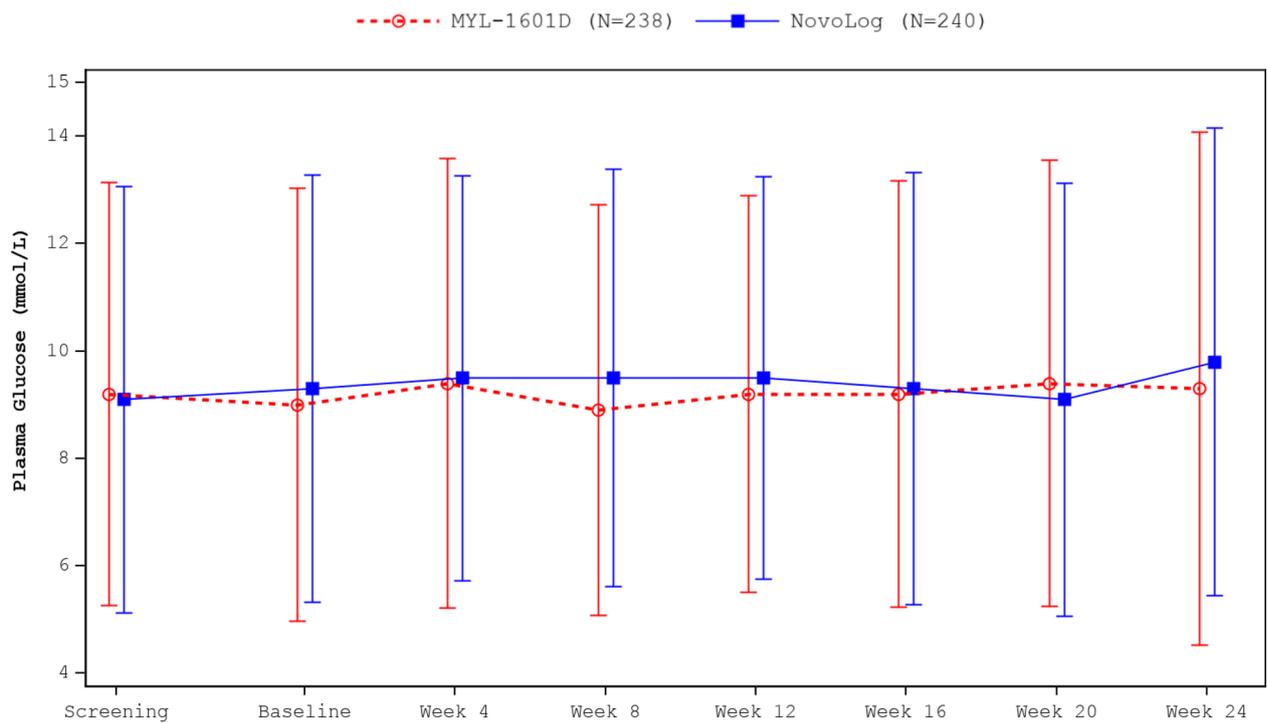
to week 24 (Fig. 2). In addition, the incidence of hypoglycemia across different categories (severe, documented symptomatic, asymptomatic, probable symptomatic, relative, nocturnal, and other hypoglycemia) were reported by a similar proportion of patients in each treatment group (Table S3, see ESM). The rate difference in the SAE of hypoglycemia was not associated with more frequent use of rescue medicine. Thus, the numerical imbalance in incidence of hypoglycemia TEAEs between the two treatment groups was not considered clinically significant.

4 Discussion

Demonstrating biosimilarity is rigorous and challenging, involving a multistep process [25]. Both European Medicines Agency and United States Food and Drug Administration (FDA) guidance on the development and approval of biosimilars requires a stepwise, totality-of-evidence-based approach to be used to generate data in support of biosimilarity and to evaluate any residual uncertainty [5, 6]. In the context of biosimilar insulin development, the FDA requirement has evolved with extensive experience and data on insulin/biosimilar availability [26]. In the EU, generally, safety studies should be performed with a specific focus on immunogenicity. However, such a study may be waived if the structural, functional, and nonclinical studies in a stepwise manner demonstrated that no clinically meaningful differences in quality, safety, or efficacy are observed compared with the reference product [27]. The approval of biosimilars like insulins need studies on immunogenicity investigation as immunogenicity measure may be correlated with product-related and process-related impurities. In this context, the clinical program of the MYL-1601D biosimilar was designed to confirm the physicochemical and functional properties, PK, PD, efficacy, safety, and immunogenicity of MYL-1601D compared with Ref-InsAsp-US/Ref-InsAsp-EU. The analytical comparison showed that MYL-1601D and reference product are highly similar molecules with respect to physicochemical and functional properties [9]. The current study was designed to address the residual uncertainty and to detect differences between the treatments groups with immunogenicity parameters [27] as the primary endpoint following demonstration of PK-PD similarity in a euglycemic clamp study, which was more sensitive to detect any difference between the biosimilar and reference product compared with HbA1C in the diabetes patients [10]. Further, the study design, including primary and secondary endpoints, population selection, treatment duration, proposed margins, and statistical assumptions, was in accordance with the US-FDA scientific advice to ensure robust patient exposure to detect differences in immunogenicity, efficacy, and safety parameters.



A



B

Fig. 1 Means and standard deviations for observed **A** HbA1c (%) values over time, **B** fasting plasma glucose (mmol/L) values over time by treatment, **C** daily mealtime insulin dose (U/kg) values over time by treatment, **D** total daily insulin dose (U/kg). Means and standard

deviations are from descriptive statistics procedure. In addition to the current product name NovoLog[®], the product code name Ref-InsAsp-US is used throughout the document. *HbA1c* glycated hemoglobin, *N* number of patients in population, *SD* standard deviation

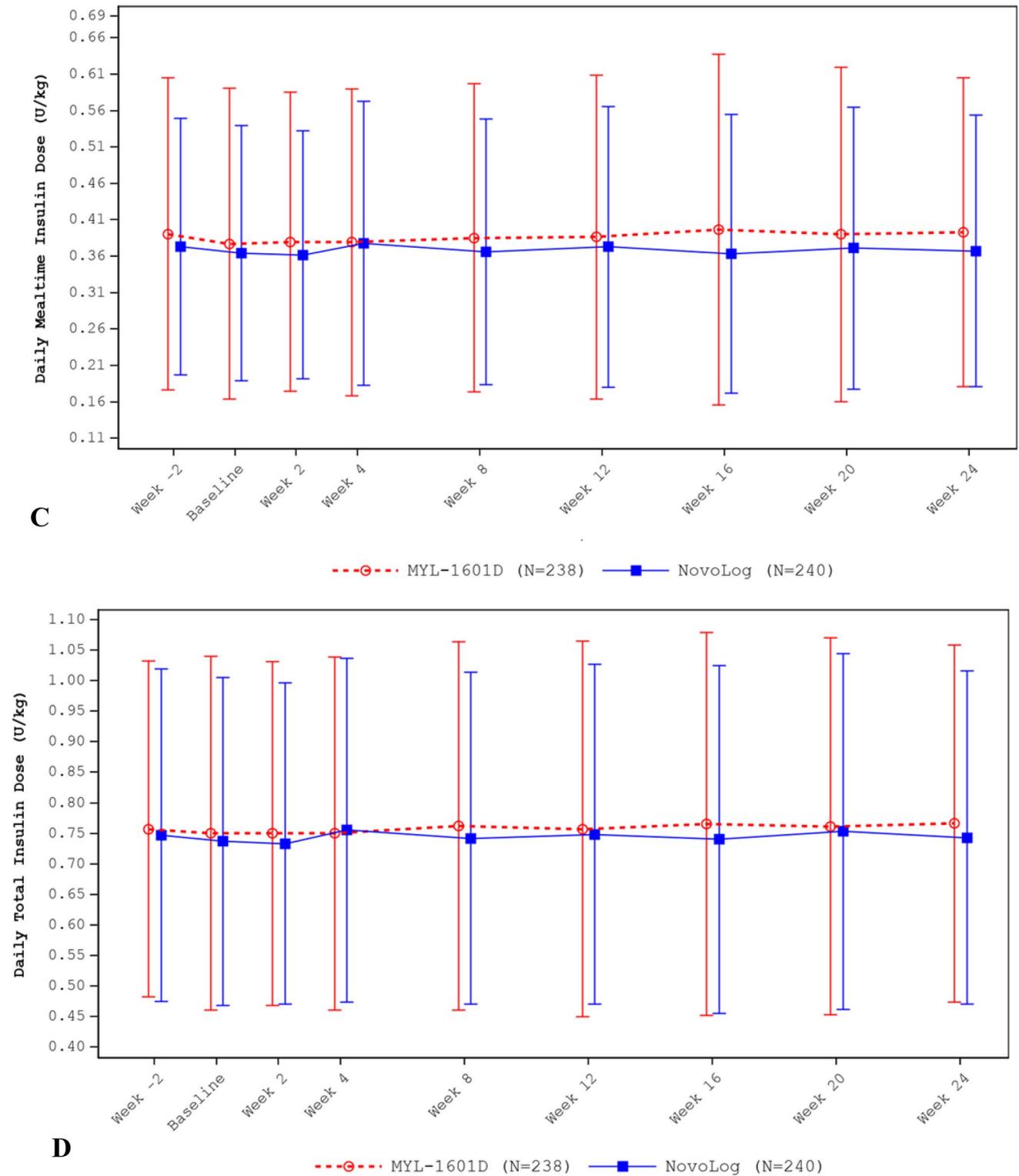


Fig. 1 (continued)

In the current study, MYL-1601D demonstrated equivalent safety and efficacy to reference Ref-InsAsp-US in patients with T1D during a 24-week treatment period. The

study met its primary objective (immunogenicity as assessed by TEAR rate) as 90% CI of the treatment difference between MYL-1601D and Ref-InsAsp-US (- 2.86 [- 9.71

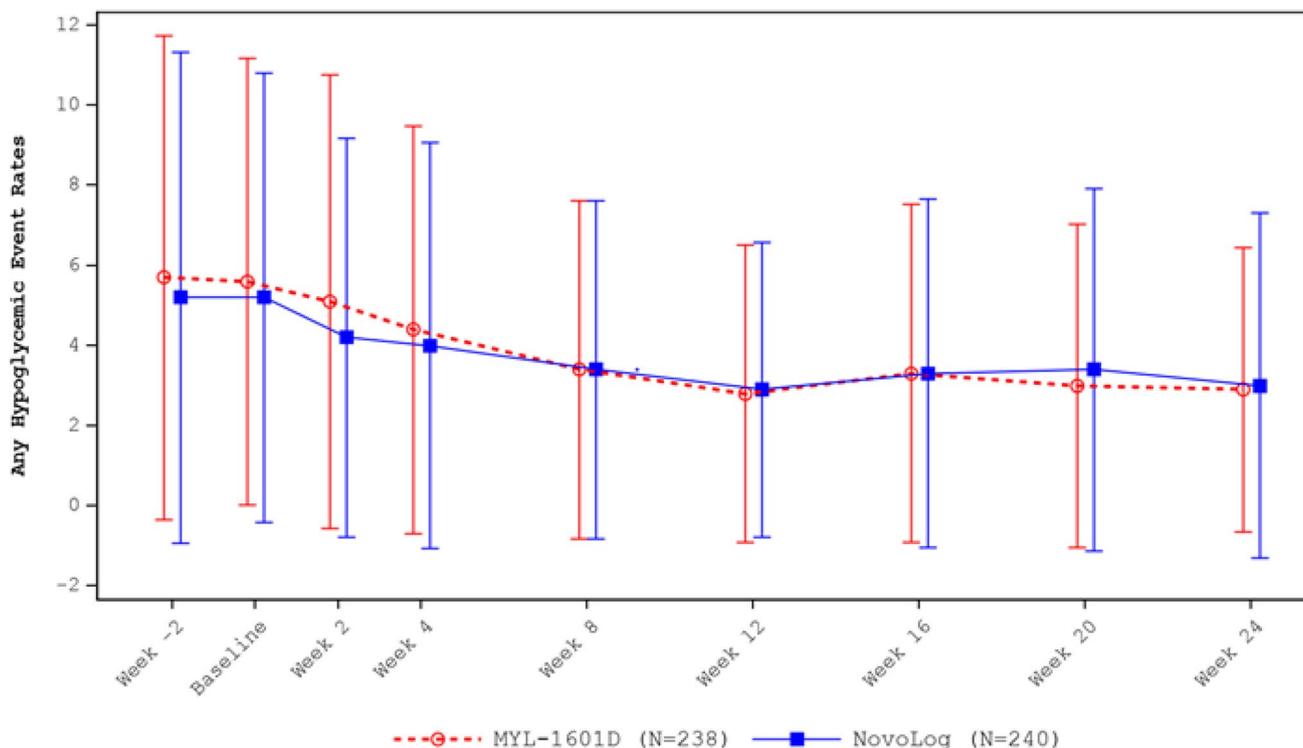


Fig. 2 Means and standard deviations for hypoglycemic event rates (episodes per 30 days adjusted). Hypoglycemia was defined as a state produced by a lower-than-normal level of glucose in the blood. In both treatment groups, the greatest reduction from baseline in hypo-

glycemic event rates was observed between baseline and week 12. Note: in addition to the current product name NovoLog[®], the product code name Ref-InsAsp-US is used throughout the document. *N* number of patients in the population, *SD* standard deviation

to 3.99]) fell within the equivalence margin of $\pm 11.7\%$. In secondary efficacy endpoints, there were no clinically meaningful changes, thus supporting the equivalent efficacy between MYL-1601D and Ref-InsAsp-US at week 24. Furthermore, sensitivity analyses were similar without imputation in a randomized set and the PP analysis set of the primary efficacy analysis, confirming the robustness of the primary endpoint results.

During the study, the percentage of patients with AIA who were cross-reactive to human insulin was similar between groups, and in line with other insulin analog studies in the T1D population [25]. Patients with AIA response, newly positive post-baseline AIA, or a 4-fold increase in AIA titer (treatment-boosted AIA) showed similar results between groups.

Safety profiles were comparable in patients with T1D between treatment groups for up to 24 weeks of treatment. Less than 10% of patients reported SAEs (MYL-1601D, 9.2%; Ref-InsAsp-US, 6.3%) with hypoglycemia being the most frequently reported SAE in both groups. The TEAE (46.6% to 48.0%) and SAE (4.9% to 6.8%) incidences were comparable to the previous reports for insulin aspart biosimilar in the T1D population [28]. A total of 15 (6.3%) patients in the MYL-1601D group and 10 (4.2%) patients in the Ref-InsAsp-US

group reported SAEs of hypoglycemia. However, it was noted that for most of the SAEs, other factors led to hypoglycemia and the causality was not related to the study drug. Notably, the incidence of severe hypoglycemic events (15 [6.3%] patients in each treatment group) in the current study was similar between both groups and was much less than the 17% reported for Ref-InsAsp-US in an RCT [29]. Furthermore, the incidence of hypoglycemic events in this study was similar to the events reported recently for an insulin aspart biosimilar and innovator product insulin aspart (at least one incidence of hypoglycemic event in 96.3–96.7% of patients, severe hypoglycemia in 3.4–4.0% patients) [15, 27].

A possible limitation of the study was that the trial was conducted in an open-labeled manner as the two products had distinct packaging. However, to avoid potential bias, the evaluation of the critical endpoints, immunogenicity, HbA1c, and FPG were analyzed at a central laboratory in a blinded manner.

5 Conclusions

We conclude that the current study demonstrates equivalent and comparable immunogenicity of MYL-1601D to Ref-InsAsp-US, as assessed by the TEAR rate during 24 weeks

of treatment. The immunogenicity findings had no clinically significant impact on both efficacy and safety parameters between the two treatment groups. Overall, MYL-1601D and Ref-InsAsp-US when given in combination with insulin glargine in patients with T1D were found to have similar efficacy and safety profiles and were well tolerated for 24 weeks.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40259-022-00554-6>.

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Declarations

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Conflict of interest Thomas C. Blevins has received clinical research support from AstraZeneca, Eli Lilly, Lexicon, Merck, Mylan, Novo Nordisk, Sanofi, Mannkind, Medtronic, and Tandem and speaker fees from Amgen, AstraZeneca, Boehringer-Ingelheim, Janssen, Eli Lilly, Merck, Novo Nordisk, and Sanofi. Bin Sun, Charles Donnelly, Roxann Shapiro, Gopinath Ranganna, and Abhijit Barve are employees of Viatris Inc. and may hold stock or stock options in the company. Yaron Raiter is an ex-employee of Viatris Inc and currently associated with GE Healthcare. Anoop Chullikana and Anita Rao are Biocon Biologics Ltd employees and may hold stock or stock options in the company. Laxmikant Vashishta is an ex-employee of Biocon Biologics Ltd. and currently a full-time employee of Alvogen Pharma India Pvt. Ltd.

Ethics approval The conduct of this study was in accordance with the ethical principles of the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines, and the appropriate regulatory requirements in the countries in which the study was conducted. The protocol and its amendments and informed consent documentation were reviewed and approved by the institutional review board(s) or independent ethics committee(s) at each study site.

Consent to participate All participants voluntarily consented to participate and signed an Institutional Review Board/Independent Ethics Committee approved informed consent form prior to study inclusion.

Consent for publication Not applicable.

Availability of data and material The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Author contributions TCB, YR, BS, CD, RS, AC, GR, and AB contributed to the conception and design of the study; acquisition, analysis, and interpretation of data; and critically revising the manuscript for important intellectual content. YR and GR contributed to the analysis and interpretation of data; drafting of the manuscript; and critically revising the manuscript for important intellectual content. CD, AR, and

LV contributed to acquisition, analysis, and interpretation of data. All authors reviewed and approved the manuscript for publication.

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