10 December 2020
EMA/64677/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kixelle

International non-proprietary name: insulin aspart

Procedure No. EMEA/H/C/004965/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Administrative information

Name of the medicinal product:	Kixelle
Applicant:	Mylan IRE Healthcare Limited Unit 35/36 Grange Parade Baldoyle Industrial Estate Dublin 13 IRELAND
Active substance:	Insulin aspart
International Non-proprietary Name/Common Name:	Insulin aspart
Pharmaco-therapeutic group (ATC Code):	insulins and analogues, insulins and analogues for injection, fast-acting (A10AB05)
Therapeutic indication(s):	Kixelle is indicated for treatment of diabetes mellitus in adults, adolescents and children aged 1 year and above.
Pharmaceutical form(s):	Solution for injection
Strength(s):	100 U/ml
Route(s) of administration:	Subcutaneous use and intravenous use
Packaging:	cartridge (glass) in pre-filled pen and vial (glass)
Package size(s):	1 pre-filled pen, 10 (2 x 5) pre-filled pens (multipack), 10 pre-filled pens, 5 pre-filled

	pens, 1 vial, 5 (5 x 1) vials (multipack) and 5 vials

Table of contents

List of abbreviations

Abbreviation	Definition
ADA	Anti-Drug Antibody
AE	Adverse Event
ANOVA	Analysis of variance
$AUC_{0\text{-}12h}$	Area under the insulin aspart concentration-time curve from 0 to 12 hours
$AUC_{GIR,0\text{-}last}$	Area under the glucose infusion rate curve from 0 hours until the end of clamp
$AUC_{GIR,0\text{-}4h}$	Area under the glucose infusion rate curve from 0 to 4 hours
$AUC_{GIR,0\text{-}6h}$	Area under the glucose infusion rate curve from 0 to 6 hours
$AUC_{GIR,6\text{-}last}$	Area under the glucose infusion rate curve from 6 hours until the end of clamp
$AUC_{0\text{-}4h}$	Area under the insulin aspart concentration-time curve from 0 to 4 hours
$AUC_{0\text{-}6h}$	Area under the insulin aspart concentration-time curve from 0 to 6 hours
$AUC_{6\text{-}12h}$	Area under the insulin aspart concentration-time curve from 6 to 12 hours
$AUC_{0\text{-}\infty}$	area under the insulin aspart concentration-time curve from 0 to infinity
B/T	Bound/Total
CFR	Code of Federal Regulation
$C_{ins.max}$	Maximum Insulin Concentration
C_{max}	Maximum Concentration
CI	Confidence Interval
CSR	Clinical Study Report
DS	Drug Substance
ECG	Electrocardiogram
EMA	European Medicines Agency
EU	European Union
FBG	Fasting blood glucose
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIR	Glucose Infusion Rate
GIR_{max}	Maximum Glucose Infusion Rate
HbA_{1c}	Glycosylated Hemoglobin
ICH	International Council for Harmonisation
IDF	International Diabetes Federation

ITT	Intent To Treat
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometric Detection
MMRM	Mixed-Effect Repeated Measure
NA	Not Applicable
PD	Pharmacodynamic(s)
PI	Prescribing information
PK	Pharmacokinetic(s)
PP	Per Protocol
PT	Preferred Term
RIPA	Radio Immunoprecipitation Assay
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous(ly)
SMBG	Self-Monitored Blood Glucose
SmPC	summary of product characteristics
$t_{1/2}$	Half-Life
λ_z	Terminal elimination rate constant of insulin
$t_{GIR,50\%\text{-early}}$	Time to half-maximum glucose infusion rate before GIR_{max}
t_{max}	Time to Reach Maximum Concentration
$t_{50\%\text{-early}}$	Time to half-maximum before C_{max}
$t_{50\%\text{-late}}$	Time to half-maximum after C_{max}
$t_{GIR,50\%\text{-late}}$	Time to half-maximum glucose infusion rate after GIR_{max}
$t_{max.GIR}$	Time to maximum glucose infusion rate
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TEAE	Treatment-Emergent Adverse Event
TEAR	Treatment emergent antibody response
US	United States
WMA	World Medical Association

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Mylan IRE Healthcare Limited submitted on 11 October 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Kixelle, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

Kixelle is indicated for treatment of diabetes mellitus in adults, adolescents and children aged 1 year and above.

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC – relating to applications for a biosimilar medicinal products

The application submitted is composed of administrative information, complete quality data, appropriate non-clinical and clinical data for a similar biological medicinal product.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: NovoRapid 100 Units/ml Solution for injection
- Marketing authorisation holder: Novo Nordisk A/S
- Date of authorisation: 07-09-1999
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EMEA/H/C/000258

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: NovoRapid 100 Units/ml Solution for injection
- Marketing authorisation holder: Novo Nordisk A/S
- Date of authorisation: 07-09-1999
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EMEA/H/C/000258

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: NovoRapid 100 Units/ml Solution for injection
- Marketing authorisation holder: Novo Nordisk A/S

- Date of authorisation: 07-09-1999
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EMEA/H/C/000258

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Scientific advice

The applicant received the following Scientific advice on the development relevant to the present application:

Date	**Reference**	**SAWP co-ordinators**
14 September 2017	EMA/CHMP/SAWP/568197/2017	*Dr Elmer Schabel, Dr Karl-Heinz Huemer*
18 October 2018	EMA/CHMP/SAWP/701744/2018	*Prof Andrea Laslop, Dr Kolbeinn Gudmundsson*
28 March 2019	EMA/CHMP/SAWP/182017/2019	*Dr Juha Kolehmainen, Dr Kolbeinn Gudmundsson*

The Scientific advice pertained to the following quality, non-clinical, and clinical aspects:

- Appropriateness of the proposed process and product comparability
- Control strategy for residual product and process related impurities as well as the need to investigate impurities in clinical safety and immunogenicity studies
- Acceptability of the plans on safe and effective use of the pre-filled Pen presentation
- Appropriateness of the planned compatibility testing to support use in insulin pump systems for continuous subcutaneous insulin infusion (CSII)
- Appropriateness of the plans to demonstrate biosimilarity based on physicochemical and functional characterisation and demonstration of PK/PD equivalence based on a euglycaemic clamp study in healthy volunteers

- Appropriateness of the design of the planned three-arm euglycaemic clamp study to demonstrate PK/PD equivalence between EU authorised NovoRapid and US-licensed NovoLog and the candidate biosimilar product
- Acceptability of the plans to characterise immunogenicity - need for a clinical safety and immunogenicity study to support benefit/risk assessment

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Kirstine Moll Harboe Co-Rapporteur: Agnes Gyurasics

CHMP Peer reviewer: Martina Weise

The application was received by the EMA on	11 October 2019
The procedure started on	31 October 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	20 January 2020
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	20 January 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	27 January 2020
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	27 February 2020
The applicant submitted the responses to the CHMP consolidated List of Questions on	14 August 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	9 October 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	1 October 2020
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	15 October 2020
The applicant submitted the responses to the CHMP List of Outstanding Issues on	10 November 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	4 December 2020

The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Kixelle on	10 December 2020
The CHMP adopted a report on similarity of Kixelle with Amglidia on (Appendix 1)	10 December 2020

2. Scientific discussion

2.1. Introduction

Problem statement

Kixelle (insulin aspart, also referred to in this report as MYL-1601D) is a human insulin analog produced by recombinant DNA technology. In EU, MYL-1601D solution for injection was developed as a biological product similar to NovoRapid (insulin aspart 100 U/mL), used as the reference product and approved in the EU through the centralised procedure on 7 September 1999. NovoRapid is indicated to improve glycaemic control in adults and children with diabetes mellitus. The Applicant is seeking approval for the same indication as NovoRapid.

About the product

MYL-1601D is produced by recombinant DNA technology utilizing Pichia pastoris (yeast). The primary structure identical to endogenous insulin, except for replacement of a single proline amino acid at position 28 in the C-terminal area of the insulin B-chain with an aspartic acid residue.

MYL-1601D drug product is qualitatively and quantitatively identical to the formulation of reference medicinal product NovoRapid with identical excipients and concentrations. MYL-1601D is presented in prefilled pens (containing 3 ml equivalent to 300 units), as well as in vials (contains 10 ml equivalent to 1000 units). 1 ml solution of MYL-1601D contains 100 units insulin aspart (equivalent to 3.5 mg).

Pharmacotherapeutic group: Drugs used in diabetes. Insulins and analogues for injection, fast-acting. ATC code: A10AB05.

The active substance in MYL-1601D 100 U/mL solution for injection is insulin aspart, a rapid-acting insulin analogue, administered subcutaneously by injection in the abdominal wall, the thigh, the upper arm, the deltoid region or the gluteal region. The blood glucose lowering effect of insulin aspart is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver. Insulin Aspart is also used for intravenous administration by physicians or other healthcare staff as applicable. Insulin Aspart can be used for continuous subcutaneous insulin infusion (CSII) in pump systems.

Type of Application and aspects on development

This Marketing Authorisation Application is an abridged application for a similar biological medicinal product under Article 10 (4) of Directive 2001/83/EC as amended by Directive 2004/27/EC. MYL-1601D has been developed as a biosimilar to the reference product NovoRapid (Insulin aspart 100 U/ml).

The clinical development programme of MYL-1601D has specifically considered the following EU guidelines:

• "Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)" (EMA/CHMP/BWP/247713/2012)

• "Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues" (EMEA/CHMP/BMWP/42832/2005 Rev. 1)

• "Annex to guideline on similar biological medicinal products containing Biotechnology-derived proteins as active substance: Non-clinical and clinical issues Guidance on similar medicinal products containing recombinant human soluble Insulin" (EMEA/CHMP/BMWP/32775/2005 Rev.1).

Based on the Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1) and Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues (EMEA/CHMP/BMWP/32775/2005_Rev. 1), the clinical program was designed to confirm the PK, PD, efficacy, safety and immunogenicity of MYL-1601D compared with NovoRapid®. The clinical development program for this MAA includes the following:

- A comparative PK/PD study in NHV, Study MYL-1601D-1001, demonstrated PK and PD equivalence between MYL-1601D and NovoRapid® and NovoLog®.
- A comparative clinical study in T1DM, Study MYL-1601D-3001 to compare the safety, immunogenicity and efficacy of MYL-1601D vs. NovoLog® .

The development programme is considered in accordance with EMA/CHMP guidelines.

2.2. Quality aspects

2.2.1. Introduction

Insulin aspart is a rapid-acting insulin with faster absorption and activity than human insulin. Kixelle is being developed as a similar biological product to NovoRapid (Insulin aspart 100 U/ml).

The finished product is presented as a solution for injection and contains 100 units/ml of insulin aspart.

Other ingredients are: metacresol, phenol, glycerol, zinc chloride, disodium phosphate dihydrate, sodium chloride, sodium hydroxide (for pH adjustment), hydrochloric acid (for pH adjustment) and water for injection.

The product is available in 10 mL vials and in a disposable pre-filled pen containing 3 mL glass cartridges and as follows:

- 10 ml solution in vial (type 1 glass) closed with a bromobutyl rubber stopper and aluminium flipoff seal. Pack sizes of 1, or 5 vials or a multipack containing 5 (5 packs of 1) vials;
- 3 ml solution in cartridge (type 1 glass) with a plunger and stopper (bromobutyl) and aluminium seal contained in a multidose pre-filled pen. Pack sizes of 1, 5, 10 pre-filled pens, or a multipack containing 10 (2 packs of 5) pre-filled pens.

2.2.2. Active Substance

General information

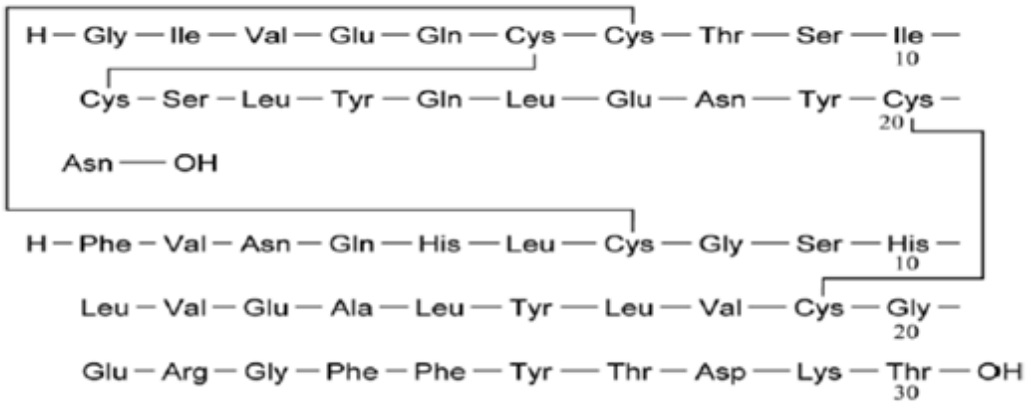
The rINN of the active substance is insulin aspart. Insulin aspart is identical in primary structure to human insulin, except that it has an aspartic acid instead of proline at position 28 of the B-chain. This change helps the fast-acting insulin analog to be absorbed quickly into the bloodstream and helps to lower the blood glucose level. The primary and secondary structure of the active substance is in agreement with the structure of insulin aspart described in the European monograph for Insulin Aspart. The molecular formula of insulin aspart is $C_{256}H_{381}N_{65}O_{79}S_6$ and the relative molecular mass is 5825.6 Da.

The amino acid sequence of A and B chain in one-letter-code and in three-letter-code with indicated disulphide bonds is presented below in Figure 1.

Figure 1: Amino acid sequence of A and B chain of insulin aspart

A Chain: GIVEQCCTSICSLYQLENYCN

B Chain: FVNQHLCGSHLVEALYLVCGERGFFYTDKT



Manufacture, process controls and characterisation

The active substance (AS) is manufactured at Biocon Sdn. Bhd., Malaysia.

Description of manufacturing process and process controls

The active substance manufacturing process has been adequately described. Main steps are fermentation, recovery and purification. The ranges of critical process parameters and the routine in-process controls along with acceptance criteria, are described for each step. The active substance manufacturing process is considered acceptable.

The yeast strain *Pichia pastoris* is used to express the recombinant insulin aspart precursor, which is cleaved and purified through downstream processes to insulin aspart, the active substance. Fermentation of the *P. pastoris* cells is conducted in a production fermenter and harvest culture supernatant captured through chromatography step followed by enzymatic cleavage step and purified by series of chromatography steps to

remove and reduce process and product related impurities. The purified MYL-1601D protein is crystalised, freeze dried and stored appropriately.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. No human or animal derived materials are used in the active substance manufacturing process and acceptable documents have been provided for raw materials of biological origin used in the establishment of cell substrate.

The gene construct was transformed into the *P. pastoris* host cell with correct insertion in the host cell genome being confirmed.

A two-tiered cell bank system, comprising of master cell bank (MCB) and working cell bank (WCB) has been established and sufficient information is provided regarding testing of MCB and WCB and release of future WCBs. The MCB and WCB are well characterized, and method description or qualification of the methods used for characterization have been provided. End of production cell bank (EPCB) and Post-production cell bank (PPCB) have been analyzed and provided evidence of genetic stability during the entire production time. A protocol for establishment of new WCBs has been provided.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests.

Appropriate hold times have been established for process intermediates and processing solutions.

Process validation

The active substance manufacturing process has been validated adequately. Consistency in manufacturing process has been demonstrated by sufficient number of full scale commercial process validation (PV) batches. A study to demonstrate the process/product related impurity clearance capability has also been conducted, confirming the reduction/clearance of several impurities by end of each chromatographic step and in the final AS for the PV batches.

Media and buffers used in manufacturing of the PV batches have been verified to be within acceptance criteria. Hold time for media and buffers, have also been established. Reusability and maximum lifetime of resins used in the chromatographic steps have been established in a small-scale study, and concurrent resin lifetime will be evaluated.

All acceptance criteria for the critical operational parameters and acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

An ongoing process verification / continued process verification (CPV) plan has been submitted, Applicant committed to continue to monitor the process performance by collecting data to identify any possible trends or abnormalities in the manufacturing process. This was endorsed and the provided CPV plan considered acceptable.

Manufacturing process development

The commercial active substance manufacturing process developmental information been provided along with comparability assessment.
FMEA methodology was used to assess the criticality of process parameters and control strategy established following a risk based approach. Process characterization studies on down-scale models were carried out after scale-up to commercial scale manufacturing process. Representativeness of the down-scale model for commercial scale has been demonstrated. OFAT or DOE studies were performed to evaluate impact of selected potential critical process parameters (pCPPs) on critical quality attributes (CQAs), and a final list of CPP and PARs were defined.

Elucidation of structure and other characteristics

The active substance has been sufficiently characterised by physicochemical and biological state-of-the-art methods revealing that the active substance has the expected structure of insulin aspart.

The full primary structure, secondary structure and tertiary structure of insulin aspart has been confirmed.

The protein content (assay) and related substances (deamidated forms and other impurities) were measured by RP-HPLCR. Results were well within the Ph. Eur. monograph specifications for Insulin Aspart. The levels of the related substances B28 Iso Asp, total deamidated (B3+A21+B3 Iso Asp) and total other impurities in the active substance are well within the specification limits given in the Ph. Eur. monograph on Insulin Aspart.

Another HPLC method is used to separate and accurately quantitate the product variants. The two RP-HPLC methods are orthogonal methods, and comparable peak annotations have been shown.

Functional characterisation (biological activity) and cell-based bioassay (mitogenic and metabolic activity) has been performed.

In summary, the analytical results are consistent with the proposed structure and the characterisation is considered appropriate for this type of molecule.

Impurities

The presence and clearance of each identified product related variant has been analyzed , showing that the variants were reduced during the RP-HPLC process steps and that most variants are present either below detection or quantification limit (LOD and LOQ) in final AS. In-process controls or controls on the release specification with acceptable acceptance limits are in place for these impurities.

A novel proinsulin impurity due to incomplete cleavage in the respective manufacturing step has been identified in the active substance compared to the reference product. The novel impurity has been well characterised and shown to not interfere with potency of the active substance. A safety and efficacy evaluation of product related impurities in Kixelle finished product has been conducted, including a comparison to the impurity levels found in NovoRapid and NovoLog. Further details concerning the level of the novel proinsulin impurity is described under "Characterisation of impurities" for finished product (below).

The levels of deamidated and isomeric variants of insulin aspart (A21Asp, B3Asp, B3IsoAsp and B28IsoAsp) found in the active substance are controlled at release with acceptance limits defined by the Ph. Eur. monograph for Insulin Aspart.

The level of HCP, HCDNA and the solvents are controlled on the AS specification.

Specification

The active substance specification includes tests for appearance, solubility, identification, product related substances / impurities, related substances, total zinc content, loss on drying, sulfated ash, residual solvents, assay, host cell protein, host cell derived DNA, bacterial endotoxins and microbial limits: total aerobic microbial count.

The active substance specification contains the test parameters required by the Ph. Eur. monograph for Insulin Aspart and relevant ICH guidelines. The applied acceptance limits for the test parameters are set based on clinical data, batch release and shelf-life data, and in accordance with Pharmacopoeial requirements.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines. Ph. Eur. methods are used for appearance, identity by peptide mapping, loss on drying, sulphated ash and endotoxin. The USP method is used for total aerobic microbial count. Detailed description of the following methods were presented: solubility, host cell DNA detection, HCP detection , residual solvent, Assay Content for related substances, SCP, HMW species. Equivalence between the Ph. Eur. methods described in the monograph for Insulin Aspart and the In-house methods have been demonstrated.

Reference standards

No in-house primary reference standard has been established since official pharmacopeia standards are available for Insulin Aspart. In-house Working reference standards are also been established. Qualification of the working standard is considered adequate. An appropriate qualification protocol for future in-house reference standards has been provided.

Batch analysis

The batch results are within the proposed specification limits, and the results confirm consistency and uniformity of the active substance, indicating that the manufacturing process is under control.

Container closure

Sufficient detailed information on the container closure system of the AS is provided and has been shown to be appropriate.

Stability

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf-life of 36 months when stored in the proposed container at -20°C and protected from light.

Stability studies under long term (-20°C ± 5°C), accelerated (5°C ± 3°C), stressed (25°C ± 2ºC/60±5% RH) and forced degradation (temperature stress and photo stability) conditions have been performed according to the ICH guidelines.

The stability studies were conducted in accordance with *ICH Q5C, Stability testing of Biotechnological/Biological Products*.

All stability data remained within defined acceptance criteria at long-term, accelerated and stressed conditions, indicating a very stable active substance.

The photo stability study followed the ICH guideline Q1B and showed that the active substance is sensitive to light degradation.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product (Kixelle) is a sterile, aqueous, clear and colourless solution containing 100 units/mL of insulin aspart.

The product is available in 10 mL vials and in a disposable pre-filled pen containing 3 mL glass cartridges and as follows:

- 10 ml solution in vial (type 1 glass) closed with a bromobutyl rubber stopper and aluminium flip-off seal. Pack sizes of 1, or 5 vials or a multipack containing 5 (5 packs of 1) vials.
- 3 ml solution in cartridge (type 1 glass) with a plunger and stopper (bromobutyl) and aluminium seal contained in a multidose pre-filled pen. Pack sizes of 1, 5, 10 pre-filled pens, or a multipack containing 10 (2 packs of 5) pre-filled pens.

The packaging material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The excipients include the preservative m-cresol and phenol, glycerol and sodium chloride as tonicity agents and zinc chloride as stabilizer in water for injection. All the excipients are of Ph. Eur. grade.

Pharmaceutical development

In general, the development of the finished product (FP) manufacturing process is found appropriate. The finished product formulation composition was based on reference product EU-Approved NovoRapid composition. Product development activities included identification of critical excipients and parameters which could impact the product quality and stability. The formulation, optimized during the formulation development, has been used in non-clinical studies and clinical testing and for the intended commercial process.

Post process characterisation studies, material attribute and process parameters were identified as Critical Process Parameter (CPP) and Non-Critical Process Parameter (NCPP). As a result of these studies, PAR and NOR were identified for CPPs and non-CPPs during routine manufacturing of finished product.

The finished product contains the two preservatives m-cresol and phenol, and the antimicrobial effectiveness by the end of in-use stability studies has been demonstrated compliant with Ph. Eur. 5.1.3.

The vial and cartridge primary container closure systems are tested for compatibility with the finished product. For vials, the glass and rubber stopper are in direct contact with FP, and for cartridges the glass cartridge, rubber plunger stopper and lined seals are in contact with FP. Extraction studies were individually

performed with the single container closure components detecting which organic compounds and inorganic substances that may migrate into the FP formulation. The screening leachable studies confirmed product compatibility with the container closure systems. The container closure integrity of both vials and cartridges is controlled in the FP specifications and tested during stability studies using leak test as per Ph. Eur. 3.2.9.

Product compatibility and in-use stability with insulin infusion pump, infusion fluid and bag, as well as insulin syringe has been sufficiently demonstrated.

Medical Device

The pen was designed and developed by considering the relevant ISO standards.

Acceptable specifications for the pen body sub-assembly are presented. The functionality of the device is confirmed in accordance with appropriate regulatory guidance's and ISO standards. A selection of commercially available needles was applied in these studies and confirmed to comply with the requirements. These needles are also indicated in the Instructions for use (SmPC section 6.6) as suitable for Kixelle pen injector. A usability study confirmed that the Kixelle pen, when used in accordance with the Instructions for Use, could be used safely and effectively by the intended user population.

Manufacture of the product and process controls

The finished product is filled in 3 mL cartridges and 10 mL vials. The cartridges are further assembled with the pen components to Kixelle disposable pen injector. A valid QP declaration and valid GMP certificates are available.

The finished product manufacturing process has been adequately described. Main steps are dissolution of AS (solution 1), preparation of excipient solution (solution 2), mixing of solution 1 & 2, pre-filtration, sterile filtration, filling and sealing.

2Process performance qualification/process validation was conducted by manufacturing sufficient number commercial scale cartridge and vial validation batches at the commercial manufacturing site. The FP manufacturing process, starting with a single AS batch or pooling of AS batches, is considered validated.

The pen assembly process involves assembling of the FP filled cartridges with the pen components. in-process checks after each step of the assembly process assure the correct assembly of the disposable pens. For the pre-filled pens, functional performance test results confirmed appropriate device functionality.

The filter validation studies are found sufficient.

The aseptic filling process has been validated in accordance with Ph. Eur. 2.6.1.

Shipping container qualification has been conducted and found acceptable. Both pens and vials will be release to the European marked after passing the release testing.

Product specification, analytical procedures, batch analysis

The finished product specification (vial) includes tests for description, pH of solution, visible particles, subvisible particles, extractable volume, identification, product related proteins/impurities, assay, zinc, m-cresol content, phenol content, seal integrity, bacterial endotoxins, sterility. The finished product specification (3 ml cartridge) includes tests for description, pH of solution, visible particles, subvisible particles, extractable

volume, identification, product related proteins/impurities, assay, zinc, m-cresol content, phenol content, seal integrity, bacterial endotoxins, sterility, dimensions of cartridge, friction force test. The finished product specification for pre-filled pen (integrated with 3 mL cartridge) includes tests for description, cosmetic test, functional test. The battery of tests listed on the FP specifications is acceptable and in line with ICH Q6B.

A justification of the specifications, including test parameters, analytical methods and acceptance criteria, has been provided. The parameters for controlling the quality of the finished product are derived by taking pharmacopeia monographs, ICH Q6B and the release and real time stability data into consideration.

Analytical methods

The analytical methods have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Characterisation of impurities

The potential impurities in the FP are stated to be the same as those described for AS. As discussed above, a novel proinsulin impurity is detected in the active substance compared to the reference product. This novel impurity is being monitored and controlled during downstream process of the AS manufacturing process. Being a process related impurity, arising during the AS manufacturing, it does not increase during the FP manufacturing nor upon storage. Complete characterization data has been provided.

A risk assessment on elemental impurities has been provided. The risk assessment was performed in accordance with ICH Q3D.

Additionally, a risk evaluation concerning the potential risk of nitrosamines in the AS and FP manufacturing process has been provided. The risk evaluation has been performed in accordance with EMA guidance. There were no risks identified, and it can therefore be concluded that there is no possibility of nitrosamine impurities being present in either AS or FP. Confirmatory testing for presence of nitrosamine impurities in AS and FP is therefore considered to be unnecessary.

Reference standards

The reference standards for MYL-1601D finished product are the same as those used for active substance. No other product specific reference standards are used in the testing of the finished product.

Batch analysis

Batch analysis data of the vial presentation, of the cartridge and PFP presentation were provided. The batch results are all well within specification limits, and the results confirm consistency and uniformity of the product, indicating that the manufacturing process is under control.

Container closure

The finished product is filled aseptically into vials or cartridges. The 3 ml Type-I clear colourless glass cartridges are plugged with sterilized bromobutyl plunger stoppers and sealed with aluminium lined seals. Each 3 mL cartridge is integrated into a Pre-filled Pen. The PFP complies with the requirements of ISO 11608-1.

The quality of the PFP components (pen body subassembly, cartridge holder and pen cap) are appropriately controlled. The packaging components for the single entity product consist of the printed carton, Patient Instruction Leaflet and Instructions for Use.

The 10 ml glass vials comply with the requirements of the USP monographs for Type-I glass containers. The rubber stoppers comply with the requirements for Type I closures specified in USP <381> and Ph. Eur. 3.2.9. The specifications have been developed to provide assurance of the suitability of the packaging material. The glass vials closed with rubber stoppers are sealed using 13 mm, round flip-off seals with aluminium and plastic parts attached to each other.

Stability of the product

The stability results indicate that the finished product is sufficiently stable. Based on available stability data, the proposed shelf-life for Kixelle (vials and pens) of 30 months when stored at 5°C±3°C as stated in the SmPC are acceptable.

All test parameters complied with their acceptance criteria when Kixelle was stored in vials and cartridges at the recommended storage condition (5°C±3°C) for up to 30 months.

Sustained functionality of the pen device during storage has been demonstrated and the provided functionality stability data is considered sufficient to support the proposed shelf life.

In-use stability data in cartridges assembled with pens and in vials were provided. The test results confirmed adequate physicochemical and microbiological quality during use. Based on the results from the in-use stability study, the in-use storage conditions recommended for cartridges integrated in a PFP after first opening are: 28 days (4 weeks) below 30°C once opened, can be stored in refrigerator (2-8°C), do not freeze.

Based on the results from the in-use stability study, the in-use storage conditions recommended for vials after first opening are: 28 days (4 weeks) below 30°C once opened, do not refrigerate, do not freeze. A comparative forced degradation study was performed with the FP and NovoRapid. The degradation pathway and rate of degradation is found to be comparable for all parameters confirming comparable stability.

Post approval change management protocol

The Applicant applies for a PACMP regarding recycling of solvent.

Adventitious agents

No material of animal origin is used in the routine manufacture of Kixelle. This applies to the active substance and all excipients used for the manufacture of the above mentioned finished product.

An adequate control strategy is in place during manufacturing to control possible contamination of the active substance and finished product.

The adventitious agents safety evaluation is considered acceptable.

GMO

Not applicable.

Biosimilarity

An extensive biosimilar exercise was conducted to demonstrate analytical similarity of Kixelle to the EU-approved reference product NovoRapid. Additionally, analytical bridging has been performed to compare Kixelle, EU NovoRapid reference product and US NovoLog comparator.

Results from side-by-side analysis of Kixelle and the reference products as well as stand-alone analyses conducted at different times were presented.

Risk ranking of quality attributes

For the biosimilarity study, quality attributes of insulin aspart that may have impact on safety and/or efficacy were classified. Product variants such as size variants, precursors and flanked variants, charge variants, and truncated forms, and structural and functional attributes comprising protein concentration, primary and higher order structures, biological activity (determined as metabolic and mitogenic activity) and zinc content, were evaluated for analytical similarity between the biosimilar and the reference product. The quality attributes are ranked and categorised for their criticality applying risk assessment principles and tools in accordance with ICH Q8 and ICH Q9, which is considered appropriate.

Statistical approach

For those quality attributes where a descriptive statistical approach was applicable (e.g. protein content, deamidation and biological activity), evaluation of the biosimilarity has been made against a pre-defined quality range.

For those quality attributes where a statistical approach was not feasible or appropriate (e.g. higher order structure measured by X-ray crystallography or NMR), similarity assessment was based on a qualitative comparison of the analytical data/profiles, which is acceptable.

Analytical methods for demonstrating biosimilarity

Structural biosimilarity between Kixelle test product and NovoRapid reference product and the comparator NovoLog was studied by applying numerous state-of-the-art methods. Intact and reduced mass determination by HPLC/ESI-MS, reduced and non-reduced peptide mass fingerprinting, Far and Near UV-CD, FT-IR, 2D NMR, fluorescence spectroscopy (intrinsic and extrinsic), differential scanning calorimetry, dynamic light scattering, X-Ray crystallography and NMR demonstrate similarity in terms of primary (i.e., amino acid sequence), secondary, tertiary structure and molecular mass. Likewise, protein content and pI of the Kixelle test product was shown to fall into the quality range of NovoRapid reference product and to be comparable between NovoRapid and the comparator NovoLog.

A novel proinsulin impurity was detected in Kixelle. This impurity cannot be found in the reference product. No change in potency of Kixelle was observed confirming efficacy-issues of this novel impurity at enriched levels.

The applied analytical panel for comparison of size variants and product impurities/variants of Kixelle active substance is considered sufficient.

Biological activity in terms of in vitro metabolic and in vitro mitogenic activity was investigated. Binding to the IR-A, IR-B and IGF-1R receptor were evaluated by Surface Plasmon Resonance (SPR) techniques and the ability of the multiple product batches to auto-phosphorylate the IR-A and IR-B receptors was measured. For metabolic endpoints, various assays measuring Adipogenesis, Inhibition of Stimulated lipolysis as well as Glucose consumption assays were performed. The results of the similarity studies concerning the biological

activity of the product, demonstrated similar biological activity for the majority of tests for Kixelle when comparing with NovoRapid batches. A summary of the results of the analytical biosimilarity study is presented below.

Table 1: Methods and summary of results used to characterize and compare MYL-1601D, NovoRapid and NovoLog

Attributes	**Methods**	**Key Findings**			**Remarks**
		MYL-1601D vs NovoRapid®	**NovoLog® vs NovoRapid®**	**MYL-1601D vs NovoLog®**	
Protein content	RP-HPLC assay	Highly similar	Highly similar	Highly similar	NA
Amino acid sequence	PMF	Identical	Identical	Identical	NA
	Intact mass	Identical	Identical	Identical	NA
	Reduced mass	Identical	Identical	Identical	NA
Confirmation (2nd and Higher order structure)	PMF (non-reduced)	Identical	Identical	Identical	NA
	FAR UV CD	Highly similar	Highly similar	Highly similar	NA
	Near UV CD	Highly similar	Identical	Highly similar	NA
	FTIR	Highly similar	Highly similar	Highly similar	NA
	Extrinsic	Highly similar	Highly similar	Highly similar	NA
	Intrinsic	Highly similar	Highly similar	Highly similar	NA
	DLS	Highly similar	Highly similar	Highly similar	NA
	DSC	Highly similar	Highly similar	Highly similar	NA
	X-Ray	Highly similar	Highly similar	Highly similar	NA
	NMR	Highly similar	Highly similar	Highly similar	NA
Size variants: Aggregated/HMWP	SEC-HPLC	Similar	Highly similar	Similar	The HMWP values in large number of MYL-1601D batches are outside the quality range. This is attributed to the relatively young age of these batches in comparison to the age of the EU approved Novorapid and US licensed NovoLog. In MYL-1601D batches at 24 months age, the HMWP values fall within the quality ranges. Hence, MYL-1601D is similar to EU-approved NovoRapid.
	SEC-MALLS	Highly similar	Highly similar	Highly similar	NA

	AUC	Highly similar	Highly similar	Highly similar	NA
pI Variant	cIEF	Highly similar	Highly similar	Highly similar	NA
Zinc content	AAS	Similar	Highly similar	Highly similar	It is noted that the values of two Kixelle batches fall below the lower limit of the quality range of NovoRapid. The marginally lower zinc content in the two batches has however been evaluated to not have an impact on their structure or function, and the difference in Zinc content observed for the two Kixelle batches does not pose any residual uncertainty for analytical similarity when compared to NovoRapid.
Truncated forms: Des B30	RP-HPLC	See remarks	Highly similar	See remarks	The levels of Des B30 in NovoRapid are below the quantitation limit of the method and hence no quantitative comparison was performed. The levels of this species seen in MYL-1601D batches does not pose a risk to patient safety and efficacy, as this is a common metabolite of insulin found in humans.
Deamidation and isomers:A21, B3, B3Iso Asp, B28	RP-HPLC	Highly similar	Highly similar	Highly similar	100% of MYL-1601D batches are within the quality range of EU-approved NovoRapid.
Mitogenic activity	IR-A cell based phosphorylation assay	Highly similar	Similar	Highly similar	NA
	Mitogenic assay using Saos2 cells	Highly similar	Highly similar	Highly similar	NA
	IR short form((IR-A) binding kinetic assay	Highly similar	Highly similar	Highly similar	NA
	IGF1R binding assay	Highly similar	Highly similar	Highly similar	NA

Metabolic activity	IR-B cell based phosphorylation assay	Highly similar	Highly similar	Highly similar	NA
	Metabolic assay using 3T3-L1 cells	Highly similar	Highly similar	Highly similar	NA
	IR-long form(IR-B) kinetic binding assay	Highly similar	Highly similar	Highly similar	NA
	Adipogenesis assay using 3T3-L1 cells	Highly similar	Highly similar	Highly similar	NA
	Inhibition of stimulated lipolysis assay using 3T3-L1 cells.	Similar	Highly similar	Highly similar	Orthogonal tests such as phosphorylation of IR-B receptor, IR Glucose uptake and IR binding suggest that these differences are not significant and overall the metabolic activity of the product is highly similar. Moreover, the values are marginally outside the established range.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The initial major objection related to the suitability of the test methods used for assay and detection of impurities were satisfactorily resolved by the applicant providing additional data. A PACMP to introduce recycling of solvent (used in the purification steps in downstream to minimise waste was considered acceptable.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Biosimilarity between Kixelle and the reference product NovoRapid/NovoLog has been demonstrated.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

This Marketing Authorization Application (MAA) for Mylan's Insulin Aspart, MYL-1601D, is submitted as a biosimilar to EU-approved NovoRapid®. EU guideline on biosimilar products are proposing a step-wise process to support the non-clinical aspects of the MAA, where *in vitro* pharmacology studies are vital to show biosimilarity, and *in vivo* animal studies should only be performed if needed (EMEA/CHMP/BMWP/ 42832/2005 Rev1). The non-clinical studies conducted should be comparative in nature and designed to have appropriate sensitivity to detect relevant differences in the response to the similar biological medicinal product and the reference medicinal product and should not just assess the response per se (EMEA/CHMP/BMWP/ 32775/2005 Rev. 1, 2015).

In the course of development, the Applicant has completed the following nonclinical studies with MYL-1601D drug product:

- Insulin Receptor-A Binding Kinetics assay
- Insulin Receptor-B Binding Kinetics assay
- IGF-1R Binding Kinetics assay
- Insulin Receptor –A Phosphorylation assay
- Insulin Receptor –B Phosphorylation assay
- Cell based bioassay for mitogenic activity
- Cell based bioassay for metabolic activity (glucose uptake)
- Cell based bioassay for metabolic activity (inhibition of stimulated lipolysis)
- Cell based bioassay for metabolic activity (adipogenesis)
- Rabbit Potency Assays, USP <121>
- Acute Toxicology (single-dose)
- Repeat Dose Toxicology and Toxicokinetics (28-Day)

All the in vitro pharmacodynamic assays supporting MYL-1601D similarity to NovoRapid (EU approved) and NovoLog (US approved) are conducted in accordance to GLP.

Two non-clinical *in vivo* toxicity studies are included in the dossier, a single dose study in rats and a 28-day repeat dose study in rats. The toxicity studies are conducted in two facilities in India and both study reports contain GLP statements from the Indian authorities. However, the test facilities were not a part of an EU or OECD Mutual Acceptance of Data (MAD) accepted GLP monitoring program in the period the studies were conducted. Hence, the two non-clinical toxicity studies are not considered to be OECD GLP compliant studies. This is acceptable since *in vivo* toxicity studies are not pivotal for biosimilar applications according to EMA guidelines (EMEA/CHMP/BMWP/ 32775/2005_Rev.1).

2.3.2. Pharmacology

Primary pharmacodynamic studies

The Applicant has followed the recommendations of the *Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues* (EMEA/CHMP/BMWP/32775/2005_Rev. 1) regarding *in vitro* non-clinical pharmacological similarity studies. The guideline recommends comparative insulin receptor binding studies, the measurement of kinetic constants, direct receptor stimulation capability determination by measuring autophosphorylation, three metabolic activity studies and, if deemed necessary, measurement of mitogenic activity. The Applicant has performed all the required studies and the mean, standard deviation and comparability range of the tested batches of the EU reference NovoRapid, NovoLog and MYL-1601D was presented in table 3.2.1.1 below. Additionally, the *in vitro* studies are briefly summarized in the following section.

Table 3.2.1.1: Mean, standard deviation and comparability range of the tested batches of NovoRapid, NovoLog and MYL-1601D for the conducted *in vitro* assays. ((presented in Table1 below)

Table 1: Mean, Standard Deviation, and Comparability Range of Tested Batches of NovoRapid®, NovoLog®, and MYL-1601D for *in vitro* Assays

Assay	Test Article	Mean	SD	Lower Limit*	Upper Limit**
IR-A Phosphorylation (relative potency)	NovoRapid®	1.08	0.05	0.93	1.23
	NovoLog®	1.12	0.08	0.89	1.35
	MYL-1601D	1.10	0.04	1.01	1.13
IR-B Phosphorylation (relative potency)	NovoRapid®	1.08	0.10	0.76	1.39
	NovoLog®	1.09	0.08	0.86	1.32
	MYL-1601D	1.12	0.07	0.99	1.21
Glucose Uptake (relative potency)	NovoRapid®	1.11	0.17	0.61	1.60
	NovoLog®	1.07	0.12	0.70	1.43
	MYL-1601D	1.00	0.09	0.82	1.13
Adipogenesis (relative potency)	NovoRapid®	96.00	8.87	69.00	122.00
	NovoLog®	101.00	3.33	91.00	111.00
	MYL-1601D	98.00	5.80	87.00	106.00
Inhibition of Stimulated Lipolysis (relative potency)	NovoRapid®	0.96	0.11	0.64	1.27
	NovoLog®	1.05	0.08	0.81	1.28
	MYL-1601D	1.11	0.14	0.93	1.30
Mitogenic Assay (relative potency)	NovoRapid®	0.96	0.08	0.72	1.21
	NovoLog®	0.98	0.10	0.68	1.28
	MYL-1601D	1.03	0.05	0.93	1.12
IGF-1R Binding Kinetics K_D, µM	NovoRapid®	2.15	0.28	1.30	2.99
	NovoLog®	2.07	0.25	1.32	2.83
	MYL-1601D	2.29	0.22	2.04	2.68
IR-B Binding Kinetics K_D, nM***	NovoRapid®	5.65	0.49	4.14	7.10
	NovoLog®	5.75	0.64	3.84	7.66
	MYL-1601D	5.72	0.49	4.89	6.85
IR-A Binding Kinetics K_D, nM***	NovoRapid®	6.64	0.41	5.41	7.86
	NovoLog®	6.73	0.57	5.03	8.43
	MYL-1601D	6.55	0.20	6.19	6.82

Abbreviation: SD: standard deviation
*For NovoRapid® and NovoLog® samples, lower limit represents the mean minus 3 times the standard deviation while for MYL-1601D the lower limit represents the minimum measured potency value
** For NovoRapid® and NovoLog® samples, upper limit represents the mean plus 3 times the standard deviation while for MYL-1601D the upper limit represents the maximum measured potency value
***In addition to K_D, k_a and k_d were also evaluated with comparability ranges reported in Section 3.2.R.1

IR-A and IR-B binding studies

Binding studies using Biacore surface plasmon resonance (SPR) technic was performed with the objective of quantify and compare binding kinetics of MYL-1601D, NovoRapid®, and NovoLog® to either insulin receptor.

IR-A and IR-B binding studies was conducted for sufficient number of batches of MYL-1601D drug product, MYL-1601D drug substance, NovoRapid® and NovoLog®, including determination of on-off kinetics, by assessment of rate of association (k_a), rate of dissociation (k_d), and dissociation constant (K_D).

IR-A and IR-B binding studies was conducted for sufficient number of batches of MYL-1601D drug product, MYL-1601D drug substance, NovoRapid® and NovoLog®, including determination of on-off kinetics, by assessment of rate of association (ka), rate of dissociation (kd), and dissociation constant (KD).

Mean values of triplicate measures were presented as mean ± standard deviation in table 3.2.1.2 below

Table 3.2.1.2: Kinetic binding of NovoRapid, MYL-1601D and NovoLog to IR-A and IR-B (Table 26 and 27, similar to the updated table 1 and 2 from the pharmacology written summary).

Table 26 Kinetic binding of NovoRapid®, MYL-1601D and NovoLog® Batches to IR-A Receptor

Batch Details	Mean $k_a \pm$ SD (1/Ms)	Mean $k_d \pm$ SD (1/s)	Mean $K_D \pm$ SD (nM)
MYL-1601D (DS)	4.32E+5 ± 2.98E+3	2.94E-3 ± 5.54E-5	6.82 ± 0.08
NovoRapid®	4.63E+5 ± 2.88E+4	3.05E-3 ± 9.11E-5	6.64 ± 0.41
MYL-1601D (DP)	4.66E+5 ± 1.51E+4	3.05E-3 ± 1.17E-4	6.55 ± 0.20
NovoLog®	4.62E+5 ± 2.22E+4	3.10E-3 ± 1.64E-4	6.73 ± 0.57

Table 27 Kinetic binding of NovoRapid®, MYL-1601D and NovoLog® Batches to IR-B Receptor

Batch Details	Mean $k_a \pm$ SD (1/Ms)	Mean $k_d \pm$ SD (1/s)	Mean $K_D \pm$ SD (nM)
MYL-1601D (DS)	4.74E+5 ± 2.61E+4	2.67E-3 ± 5.74E-5	5.70 ± 0.15
NovoRapid®	4.74E+5 ± 3.88E+4	2.64E-3 ± 9.04E-5	5.62 ± 0.49
MYL-1601D (DP)	4.69E+5 ± 2.32E+4	2.67E-3 ± 1.41E-4	5.72 ± 0.49
NovoLog®	4.67E+5 ± 3.61E+4	2.66E-3 ± 1.21E-4	5.75 ± 0.64

For the purpose of comparability assessment, quality range approach was applied where the quality ranges were determined based upon the Mean ± 3SD (standard deviation) of all analysed NovoRapid® batches. Then, MYL-1601D was compared against the range established by NovoRapid®.

Comparability was considered demonstrated if more than 90% of the MYL-1601D batches fell within the lower and upper limit of NovoRapid® batches (Mean±3SD). In table 3.2.1.3 below, data from the comparability assessment of the IR-A and IR-B binding kinetic studies are presented.

Table 3.2.1.3: Collection of data from the comparability assessment of the IR-A and IR-B binding kinetics. Table 24 represent the summary of data for binding kinetic study using IR-A receptor and Table 25 represent the summary using IR-B receptor.

Table 24: Summary of Data Comparison amongst NovoRapid®, MYL-1601D and NovoLog® batches with respect to its kinetic constant parameters using IR-A receptor.

Batch ID	Range	k_a (1/Ms)	k_d (1/s)	K_D (nM)
NovoRapid®	Lower Limit*	3.76E+05	0.0028	5.41
	Upper Limit$	5.49E+05	0.0034	7.86
MYL-1601D	Minimum Observed Value	4.34E+05	0.0028	6.19
	Maximum Observed Value	4.87E+05	0.0032	6.82
NovoLog®	Lower Limit*	3.96E+05	0.0026	5.03
	Upper Limit$	5.29E+05	0.0036	8.43

*Mean -3SD; $Mean+3SD

Table 25: Summary of Data Comparison amongst NovoRapid®, MYL-1601D and NovoLog® batches with respect to its kinetic constant parameters using IR-B receptor.

Batch ID	Range	k_a (1/Ms)	k_d (1/s)	K_D (nM)
NovoRapid®	Lower Limit*	3.58E+05	0.0024	4.14
	Upper Limit$	5.91E+05	0.0029	7.10
MYL-1601D	Minimum Observed Value	4.39E+05	0.0025	4.89
	Maximum Observed Value	5.26E+05	0.0030	6.85
NovoLog®	Lower Limit*	3.59E+05	0.0023	3.84
	Upper Limit$	5.75E+05	0.0030	7.66

*Mean -3SD; $Mean+3SD

IR-A and IR-B phosphorylation studies

Phosphorylation of the receptors by insulin aspart were evaluated in CHO-K1 cells overexpressing IR-A and IR-B using AlphaScreen Surefire technology.

Sufficient number of batches of MYL-1601D drug product, NovoRapid® and NovoLog® were tested in assays for both IR-B and IR-A phosphorylation with the objective to quantify and compare the capacity of MYL-1601D, NovoRapid®, and NovoLog® to generate autophosphorylation.

Relative potencies were calculated by comparison to the Internal working reference standards (IWRS) and presented as mean±SD. Results are presented in Table 3.2.1.4 below.

Table 3.2.1.4: Relative potency of phosphorylation of insulin receptor A and B in response to MYL-1601D, NovoRapid and NovoLog (Table 3 and 4 from the pharmacology written summary).

Table 3: Phosphorylation of Insulin Receptor B in Response to MYL-1601D, NovoRapid®, or NovoLog®

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	1.12 ± 0.07	0.99 to 1.21
NovoRapid®	1.08 ± 0.10	0.89 to 1.23
NovoLog®	1.09 ± 0.08	0.94 to 1.22

Table 4: Phosphorylation of Insulin Receptor A in Response to MYL-1601D, NovoRapid®, or NovoLog®

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	1.10 ± 0.04	1.01 to 1.16
NovoRapid®	1.08 ± 0.06	0.98 to 1.16
NovoLog®	1.12 ± 0.08	0.96 to 1.24

Applicant concluded that the results indicates that MYL-1601D's capacity to phosphorylate insulin receptor B and A is comparable to that of NovoRapid® and NovoLog®.

Studies on metabolic activity

Three different assays on metabolic activity were conducted in accordance with the guidelines (EMEA/CHMP/BMWP/ 32775/2005_Rev.1), measuring glucose uptake/consumption, inhibition of stimulated lipolysis and lipogenesis respectively. The choice of studies is supported.

- *Glucose consumption assay*

Sufficient number of batches of MYL-1601D drug product, MYL-1601D drug substance, NovoRapid®, and NovoLog® were used for testing glucose uptake response. Samples was compared to the IWRS. Results are presented in table 3.2.1.5 below.

Table 3.2.1.5: Glucose uptake of adipocytes (table 5 from the pharmacology written summary).

Table 5: Glucose Uptake (Metabolic Potency) of Adipocytes in Response to MYL-1601D, NovoRapid®, or NovoLog®

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	1.00 ± 0.09	0.82 to 1.13
MYL-1601D DS	0.97 ± 0.09	0.87 to 1.04
NovoRapid®	1.11 ± 0.17	0.83 to 1.42
NovoLog®	1.07 ± 0.12	0.86 to 1.24

- *Inhibition of Stimulated Lipolysis*

Sufficient number of batches of MYL-1601D drug product, NovoRapid® and NovoLog® were compared in this assay for inhibition of stimulated lipolysis in differentiated 3T3-L1 cells. Samples was compared to the IWRS. Results are presented in table 3.2.1.6 below.

Table 3.2.1.6: Inhibition of stimulated lipolysis (table 7 from the pharmacology written summary).

Table 7: Inhibition of Stimulated Lipolysis (Metabolic Potency) of Adipocytes in Response to MYL-1601D, NovoRapid®, or NovoLog®

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	1.11 ± 0.14	0.93 to 1.30
NovoRapid®	0.96 ± 0.11	0.81 to 1.17
NovoLog®	1.05 ± 0.08	0.90 to 1.13

- *Adipogenesis study*

Sufficient number of batches of MYL-1601D drug product, NovoRapid® and NovoLog® were compared in this assay with the objective of assessing adipogenesis. The outcome of the sample analysis was expressed in terms of "Mean Percent Relative Potency" by comparing the sample to the IWRS. The percent relative potency of "Reference Standard" was presumed to be 100%.

Results are presented in table 3.2.1.7 below.

Table 3.2.1.7: Adipogenesis (table 6 from the pharmacology written summary).

Table 6: Adipogenesis (Metabolic Potency) of Adipocytes in Response to MYL-1601D, NovoRapid®, or NovoLog®

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	0.98 ± 0.06	0.87 to 1.06
NovoRapid®	0.96 ± 0.09	0.85 to 1.09
NovoLog®	1.01 ± 0.03	0.95 to 1.04

In vivo rabbit blood sugar potency study

The rabbit potency study was conducted in consideration of the USP <121> monograph guidelines, as an FDA requirement. However, this is not in accordance to EMA guidelines as "*comparative in vivo studies of pharmacodynamic effects would not be anticipated to be sensitive enough to detect differences not identified by in vitro assays, and are not required as part of the comparability exercise*" (EMEA/CHMP/ BMWP/32775/2005_Rev.1).

Required number of batches of MYL-1601D DS has been tested. As reference standard USP Insulin Aspart RS lot F0L097 was used (USP certificate is included in Annexure 4 of the study reports). Results are presented in table 3.2.1.8 below.

Table 3.2.1.8: Rabbit potency assay (table 7 from the non-clinical overview).

Table 7: Potency of MYL-1601D in Rabbits by USP <121>

Batch	Potency (U/mg)
USP Reference Standard Insulin Aspart (F0L097)	28.59
MYL-1601D (Mean ± Standard Deviation)	28.39 ± 1.11

The data from the rabbit blood sugar potency test has been assessed and the study has been conducted properly. However, the results of the rabbit study are considered as supplementary since *in vivo* studies are not sensitive enough for biosimilar testing and therefore not necessary according to guideline. The insensitivity of the study was even more pronounced by the fact that neither of the insulin aspart doses caused symptomatic hypoglycaemia in the fasted animals, nor was any differences seen in the blood glucose lowering potencies of the test and reference insulin aspart preparations.

Studies on mitogenic activity

According to guidelines (EMEA/CHMP/BMWP/32775/ 2005_Rev.1) an assay on comparative IGF-1 receptor binding and functional activity can be included to cover this potential toxicological effect. In the current MAA the applicant has included an IGF-1 receptor binding assay in addition to a mitogenic activity assay conducted in Saos2 cells.

Sufficient number of batches of MYL-1601D drug product, MYL-1601D drug substance, NovoRapid® and NovoLog® were assessed in these assays. Results are presented in table 3.2.1.9 below.

Table 3.2.1.9: Kinetic binding and mitogenic potency of MYL-1601D, NovoRapid and NovoLog to IGF-1R (table 9 and 10 from the pharmacology written summary).

Table 9: Kinetic Binding of MYL-1601D, NovoRapid®, and NovoLog® to IGF-1R

	Mean ± SD k_a (1/Ms)	Mean ± SD k_d (1/s)	Mean ± SD 1:1 Binding K_D (nM)
MYL-1601D DP	3.83E+4 ± 2.69E+3	8.63E-2 ± 4.95E-3	2290 ± 215
MYL-1601D DS	2.67E+4 ± 2.13E+4	9.77E-2 ± 1.11E-2	2490 ± 444
NovoRapid®	3.85E+4 ± 3.46E+3	8.29E-2 ± 6.23E-3	2160 ± 289
NovoLog®	4.00E+4 ± 3.15E+3	8.07E-2 ± 6.03E-3	2050 ± 242

Table 10: Mitogenic Potency of MYL-1601D, NovoRapid®, and NovoLog® in Saos2 IGF-1R expressing cells

	Mean ± SD (Relative Potency)	Range
MYL-1601D DP	1.03 ± 0.05	0.93 to 1.12
MYL-1601D DS	0.85 ± 0.05	0.81 to 0.90
NovoRapid®	0.96 ± 0.08	0.81 to 1.12
NovoLog®	0.98 ± 0.11	0.76 to 1.09

The rationale for carrying out these studies was that signalling through IGF-1R can potentiate growth and proliferation of cancer cell lines. This argumentation and the conduct of the studies are endorsed.

Secondary pharmacodynamic studies

No studies regarding Secondary Pharmacodynamics were performed.

Safety pharmacology programme

No studies regarding Safety Pharmacology were performed.

Pharmacodynamic drug interactions

According to the *guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1)* studies regarding safety pharmacology, reproduction toxicology, and carcinogenicity are not required for non-clinical testing of biosimilars.

No pharmacodynamic interactions was expected as insulins are very specific hormones and no other drugs act on insulin receptors. The lack of pharmacodynamic interaction studies is accepted.

2.3.3. Pharmacokinetics

No dedicated non-clinical pharmacokinetic studies were conducted in support of the current MAA for MYL-1601D. This is acceptable, as the product is intended as a biosimilar medicinal product with NovoRapid as the EU reference product. No nonclinical pharmacokinetic studies should be performed according to guidelines.

2.3.4. Toxicology

Two rat toxicity studies, a single dose acute toxicity study and a 28-day repeat dose toxicity study, was included in this biosimilar application. In both studies the US licensed NovoLog was used as reference product, not the EU approved NovoRapid.

Single dose toxicity

An iv single dose acute toxicity study in rats (Study U-16366) comparing MYL-1601D to the US-approved NovoLog was performed. No evidence of systemic toxicity was seen in the single dose toxicity study of doses approximately 20-, 40-, and 80-fold the intended therapeutic starting dose in humans.

Maximum tolerated dose in the rats were determined to be the highest tested dose of 17.5 mg/kg (500 U/kg) for both of MYL-1601D and NovoLog. The only consistent observation was symptoms of hypoglycaemia, which is an expected exaggerated pharmacology effect, following administration of insulin to normo-glycaemic animals. No reversible, persistent or delayed toxic effects was registered during the 14-day post injection observation period.

Repeat dose toxicity

A 28-day repeat-dose toxicity study in rats (Study U-16377) comparing subcutaneous injections of MYL-1601D to the US-approved NovoLog was performed. A 14-day recovery group and a toxicokinetic/pharmaco-dynamic group were included in the study and treated with similar doses as the main toxicity group. No evidence of systemic toxicity was seen in the repeat-dose toxicity study in rats with daily doses of 0.21, 0.63, or 2.1 mg/kg/day, equivalent to 1-, 3-, or 10-fold the intended starting therapeutic dose in humans (1 U/kg/day; 0.035 mg/kg/day). A NOAEL>2.1 mg/kg/day was established for both MYL-1601D and NovoLog®.

An indication of comparable pharmacodynamic and toxicokinetic effects was seen for MYL-1601D and NovoLog. Both drugs induced a reduction in blood glucose within the same interval (36-50 mg/dl) and no dose proportional increase in pharmacodynamic response were observed for either drug. Comparable drug exposure was seen for MYL-1601D and NovoLog and no signs of accumulation between day 1 and 28 were observed. A small difference was seen in when T_{max} is obtained (at 0,5 or 6,5 h). However, as two separate serum insulin peaks are seen for both drug items, it can only be concluded that T_{max} is obtained approximately 30 min after injection for both types of Insulin Aspart. An indication of a more than dose proportional increase in systemic insulin exposure and peak serum concentrations was observed on both day 1 and 28. No obvious differences were seen across sexes.

Like in the acute toxicology study, the repeat dose study also demonstrated consistent observations of hypoglycemia in both MYL-1601D and NovoLog® treated rats. Hypoglycemia is an anticipated pharmacological effect and was not considered adverse for the purpose of these toxicology studies.

In the repeat-dose toxicity study evidence of local reactions at the injection sites was detected as hyperaemia in the subcutis corresponding histologically to haemorrhage and cell infiltration with primarily mononuclear cell and occasionally neutrophil granulocytes. However, similar changes were seen in all treatment groups, including placebo treated animals. The lesions were reversible and not detected in the recovery group at day 43. It is therefore agreed, that the lesions are related to repletely induced injection trauma and independent of test or reference drugs injected.

Local tolerance endpoints were therefore successfully included in the repeat dose toxicity study performed. Since no new excipients have been included, no stand-alone non-clinical local tolerance study is required according to the relevant guidelines for similar biological pharmaceuticals (CHMP/437/ 04 Rev 1; EMEA/CHMP/BMWP42832/2005/Rev 1; and EMEA/CHMP/BWMP/322775/2005 Rev 1).

Genotoxicity

No genotoxicity studies were performed with Myl-1601D.

Carcinogenicity

No carcinotoxicity studies were performed with Myl-1601D.

Reproduction Toxicity

No reproduction toxicity studies were performed with Myl-1601D.

Local Tolerance

In the repeat-dose toxicity study evidence of local reactions at the injection sites was detected as hyperaemia in the subcutis corresponding histologically to haemorrhage and cell infiltration with primarily mononuclear cell and occasionally neutrophil granulocytes. However, similar changes were seen in all treatment groups, including placebo treated animals. The lesions were reversible and not detected in the recovery group at day 43. It is therefore agreed, that the lesions are related to repletely induced injection trauma and independent of test or reference drugs injected.

Local tolerance endpoints were therefore successfully included in the repeat dose toxicity study performed. Since no new excipients have been included, no stand-alone non-clinical local tolerance study is required according to the relevant guidelines for similar biological pharmaceuticals (CHMP/437/ 04 Rev 1; EMEA/CHMP/BMWP42832/2005/Rev 1; and EMEA/CHMP/BWMP/322775/2005 Rev 1).

Other toxicity studies

A novel proinsulin impurity due to incomplete enzymatic cleavage has been identified in MYL-1601D DS. The efficacy of the novel impurity has been sufficiently discussed in the quality part of the MAA and it is shown to not interfere with potency of MYL-1601D. A summary of the discussion of the metabolism and safety of this impurity and its precursors are provided as follows.

Metabolism of the Proinsulin Aspart Impurity

The similarity of the insulin aspart impurity sequence to sequences from endogenous human proteins was provided by the applicant. The similarity in sequences indicated that this peptide will be metabolized/eliminated in a comparable manner with the insulin aspart. Therefore, it was concluded that considering the presumed capability of the endogenous proteases to digest the insulin aspart impurity, the rapid digestion of native insulin by these enzymes, and the short half-life of proteins with similar sequences as the proinsulin impurity in the body, any administered such impurity is expected to be rapidly degraded with no accumulation.

Metabolism of Insulin aspart precursors

Even though enzymatic processing potentially could generate multiple impurities resulting from alternative trypsin cleavage sites, only the SCP (single chain precursor) and the proinsulin impurity are detected in MYL-1601D DS or DP. The release from subcutaneous compartment, the half-life and the degradation by endogenous enzymes of SCP is expected to be similar to that of proinsulin. The non-native sequence components is expected to be digested by endogenous catabolic processes as presented above.

Safety

The safety of the proinsulin impurity and SCP was supported by the repeat dose toxicology study U-16377 in rats. In this study, the rats receiving the highest dose were exposed to at least 3-4 times the expected clinical dose of the impurities without showing evidence of toxic events. The NOAEL of the study was actually set to the highest dose of 2.1 mg/kg/day, which additionally substantiates the safety of the impurities.

2.3.5. Ecotoxicity/environmental risk assessment

Insulin aspart, MYL-1601D, is a peptide, and peptides are exempted from performing any ERA studies, as they are considered to be a natural substance, and to rapidly biodegrade, and hence not pose any risk to the environment.

Furthermore, insulin aspart is already used in existing marketed products and no significant increase in environmental exposure is anticipated following marketing of Applicants product.

2.3.6. Discussion on non-clinical aspects

Pharmacology

A sufficient number of *in vitro* pharmacology studies have been performed in order to assess receptor binding, receptor phosphorylation and metabolic activity for MYL-1601D insulin aspart and NovoRapid (and NovoLog), in accordance to the relevant guideline (EMEA/CHMP/BMWP/ 32775/2005 Rev. 1, 2015). The methods used in the *in vitro* pharmacology studies are acceptable and sensitive enough to detect difference between the test and the reference compounds. The number of tested batches and the number of runs/replicates are sufficient. The batch used in the clinical study MYL-1601D-1001 was tested in all *in vitro* studies.

Minimum and maximum potency values of MYL-161D were within the lower and upper limit for the EU reference NovoRapid (and NovoLog), as presented by the applicant in table 3.2.1.1. The only exception was the upper limit of the inhibition of stimulated lipolysis which diverged slightly compared to the upper limit of NovoRapid. Overall, this was considered sufficient to confirm biosimilarity from a non-clinical point of view. It should, nevertheless, be noted that the relative potencies of the compounds were calculated toward an internal working reference standard (IWRS) instead of providing the actual data with their standard errors. The "*Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues*" (EMEA/CHMP/BMWP/ 32775/2005_Rev. 1) states: "*Biosimilar and reference product should be compared head-to-head in the same experiment.*" Using an internal standard makes the comparison between MYL1601D and NovoRapid® indirect. The rationale given to explain the use of internal standards were acceptable from a quality point of view as batch consistency is a critical quality issue. However, in non-clinical pharmacology the test and reference batches used in the studies must be compared with each other. In the clinical studies, no internal reference standard is used, the test compound and the EU reference are compared directly and non-clinical *in vitro* pharmacology studies should follow the same approach. Given that in vitro studies are generally more sensitive than clinical trials direct comparisons can reveal differences better. If the test and reference compounds give the same results (meaning there is no significant differences between the final parameters of each in vitro study) the test compound is biosimilar to the reference from a non-clinical pharmacology point of view.

However, EC50/IC50 values of the functional *in vitro* pharmacology studies were provided and showed visual overlap of EC50 values of MYL-1601D, NovoRapid, NovoLog and IWRS in the different assays. Additionally, individual concentration-effect curves of each biological activity assay, all showed the classic sigmoid shape and the test compound curve overlapped with that of the IRWS. Furthermore, the two IWRS, i.e. BS16003812 and BS17008947, have been sufficiently characterized in order to assess comparability between MYL-1601D, NovoRapid® and NovoLog®. Therefore, the totality of the data provided was considered sufficient to support non-clinical biosimilarity and the issue of the lacking head-to-head comparison will not be further pursued.

Toxicology

Two rat toxicity studies, a single dose acute toxicity study and a 28-day repeat dose study, were included in this biosimilar marketing authorisation application.

Generally, *in vivo* studies are considered not to be sufficiently sensitive to conclude on biosimilarity. Consequently, *in vivo* toxicity studies are not a requirement according to EMA guidelines and hence the animal studies are unnecessary from a 3R perspective (EMEA/CHMP/BMWP/32775/2005 _Rev.1). *In vivo* studies should only be conducted if uncertainties of safety issues remain after the step-wise approach based on the result of extensive structural and functional characterization (EMEA/CHMP/BMWP/42832/2005 Rev1). Furthermore, it should be mentioned that the US-approved NovoLog has been used as reference product in the toxicity studies instead of the EU-approved NovoRapid.

The two toxicity studies are only used for guidance on maximum tolerated doses (MTD) and indications of comparable reaction pattern between MYL-1601D and NovoLog/NovoRapid. In conclusion, the studies are considered to be of supplementary character and not pivotal for approval, since they are not sufficiently sensitive for biosimilar assessment, as specified in the relevant guidelines and previously highlighted.

Other toxicity studies

A novel proinsulin aspart impurity, was identified in Kixelle but not in the reference product. The impact of this impurity on efficacy was addressed in the quality part of the assessment. A sufficient discussion of the metabolism of this impurity and insulin aspart precursors and relevant literature was presented in the non-clinical material. Furthermore, the safety of this impurity and the single chain precursor was supported by the repeat dose toxicology study U-16377, where the rats received 3-4 times the amount of the impurities as expected in the human clinical setting.

2.3.7. Conclusion on the non-clinical aspects

Providing evidence of pharmacological similarity between the product under review and the reference product is the main objective of a biosimilar application. In the current MAA, non-clinical biosimilarity has been confirmed between MYL-1601D and the EU reference NovoRapid (and NovoLog) based on *in vitro* studies for receptor binding and metabolic activity in accordance with ICH guidelines.

SmPC wording in section 4.6 and 5.3 is identical to the reference product SmPC and therefore acceptable.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 4 List of pivotal and supportive clinical studies (completed/ongoing) for MYL-1601D

Clinical studies	Study elements	
Completed Study		
MYL-1601D-1001 (pivotal): Phase 1 PK / PD equivalence; euglycemic clamp study; NHV (Germany)	Study design	Randomized, double blind, three-treatment, three-period crossover euglycemic glucose clamp trial
	Source of reference product	EU-approved reference product and US-licensed comparator product
	Sample size and study population	Overall, 71 subjects (NHV) were exposed to IMP, 70 to MYL-1601D, 69 to NovoLog, and 67 to NovoRapid. Dose of each product was 0.2 U/kg body weight.
Ongoing Study		
MYL-1601D-3001 (supportive): Phase 3 safety, immunogenicity and efficacy study in T1DM (US)	Study design	open-label, randomized, parallel-group phase 3 study
	Source of reference product	US-licensed comparator product
	Sample size and study population	Approximately 500 subjects with T1DM will participate in this trial.

NHV: normal healthy volunteers; PD: pharmacodynamic(s); PK: pharmacokinetic(s); T1DM: type 1 diabetes mellitus

2.4.2. Pharmacokinetics

Study design

Study MYL-1601D-1001 was a randomised, double-blind, three-treatment, three-period, crossover, 12-hour euglycemic glucose clamp study that was conducted in order to demonstrate biosimilarity between MYL-1601D and NovoRapid (EU-approved) and NovoLog (US-licensed), respectively. A single dose of 0.2 U/kg was used, and the blood glucose concentrations were clamped at 81mg/dl (4.5 mmol/l). Several blood samples were collected during the clamp. There were 12-16 days between the clamp visits.

Plasma insulin aspart concentration profiles were used to calculate the PK parameters.

Primary PK endpoints:

- $AUC_{0\text{-}12h}$, area under the insulin aspart concentration-time curve from 0 to 12 hours
- C_{max}, maximum observed insulin aspart concentration

Secondary PK endpoints:

- $AUC_{0\text{-}4h}$, area under the insulin aspart concentration-time curve from 0 to 4 hours
- $AUC_{0\text{-}6h}$, area under the insulin aspart concentration-time curve from 0 to 6 hours
- $AUC_{6\text{-}12h}$, area under the insulin aspart concentration-time curve from 6 to 12 hours
- $AUC_{0\text{-}\infty}$, area under the insulin aspart concentration-time curve from 0 to infinity
- t_{max}, time to maximum observed insulin aspart concentration
- $t_{50\%\text{-}early}$, time to half-maximum before C_{max}
- $t_{50\%\text{-}late}$, time to half-maximum after C_{max}
- $t_{½}$, the terminal elimination half-life calculated as $t_{½}=\ln 2/\lambda z$
- λz, terminal elimination rate constant of insulin

Three separate analysis of variance (ANOVA) models were conducted – excluding the data from the treatment that was not part of the comparison. The primary PK endpoints, derived from plasma insulin aspart concentrations profiles, were analyzed using logarithm-transformed data. The ANOVA was based on a general linear model (proc GLM) using clinical site, treatment, sequence, period, clinical site by sequence, clinical site by treatment, and subject within (clinical site by sequence) as fixed effects. Clinical site by treatment interaction was dropped from the analysis since not found to be significant. Data was logarithmically transformed and the 90% CI for the endpoints were calculated and evaluated according to the 80%-125% pre-specified limits. The per protocol population was used for PK analysis.

The study population consisted of healthy men and women. 72 subjects were randomised. One subject withdrew before first dose, and three subjects withdrew their consent during the study. Two subjects did not meet the required time window in terms of scheduling the visits. 70 subjects were exposed to MYL-1601D, 69 to Novolog and 67 to NovoRapid. Consequently, the per protocol populations for the MYL-1601D vs. NovoLog and MYL-1601D vs. NovoRapid comparisons consist of 68 and 67 subjects, respectively.

Pharmacokinetic results

For the primary endpoints, $AUC_{(0\text{-}12h)}$ and C_{max}, the 90% confidence intervals were within the predefined limits (80% and 125%) for the comparison with NovoLog as well as NovoRapid (Figure 2 and Table 3).

For one of the secondary endpoints, $AUC_{(6\text{-}12h)}$ the ratio between MYL-1601D and NovoLog was 75.60 (61.38; 92.16), and the ratio between MYL-1601D and NovoRapid was 81.37 (62.70; 105.75) and outside the predefined limits of biosimilarity.

The mean $T_{½}$ for MYL-1601D, NovoRapid and NovoLog were 0.88 h, 0.89 h and 0.93 h, respectively, and are considered similar.

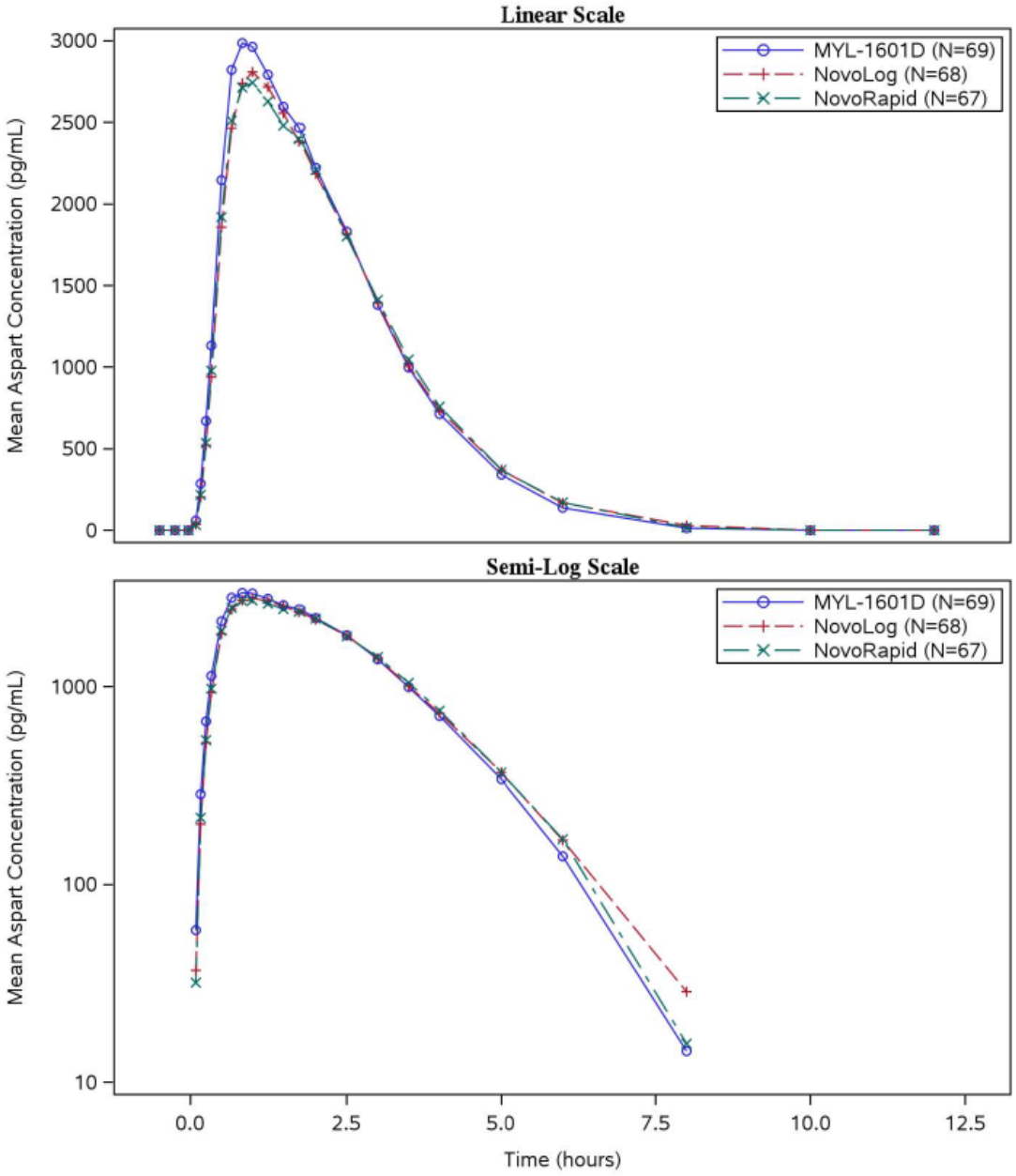


Figure 2 **Mean Insulin Aspart Concentration vs. Time by Treatment (Linear and Semi-Log Scale)**

Cross-reference: EOT Figure 14.2.2.2

Table 1: Treatment comparisons for pharmacokinetic endpoints (PPP for PK)

Parameter	Comparison	N	Ratio %[3]	90% CI[4]	CV%[5]
AUC_{0-12h} (h*pg/mL)[1]	**MYL-1601D vs. NovoLog®**	**68/68**	**102.17**	**(100.26; 104.11)**	**6.6**
	MYL-1601D vs. NovoRapid®	**67/67**	**101.84**	**(100.04; 103.67)**	**6.2**
	NovoLog® vs. NovoRapid®	66/66	99.67	(97.96; 101.40)	5.9
C_{max} (pg/mL)[1]	**MYL-1601D vs. NovoLog®**	**68/68**	**106.13**	**(100.71; 111.85)**	**18.5**
	MYL-1601D vs. NovoRapid®	**67/67**	**105.74**	**(101.09; 110.60)**	**15.7**
	NovoLog® vs. NovoRapid®	66/66	99.89	(95.46; 104.53)	15.7
AUC_{0-4h} (h*pg/mL)[2]	MYL-1601D vs. NovoLog®	68/68	104.24	(101.42; 107.11)	9.5
	MYL-1601D vs. NovoRapid®	67/67	104.30	(101.74; 106.86)	8.6
	NovoLog® vs. NovoRapid®	66/66	100.23	(97.79; 102.68)	8.3
AUC_{0-6h} (h*pg/mL)[2]	MYL-1601D vs. NovoLog®	68/68	103.23	(101.14; 105.34)	7.1
	MYL-1601D vs. NovoRapid®	67/67	102.90	(100.93; 104.84)	6.7
	NovoLog® vs. NovoRapid®	66/66	99.71	(97.90; 101.52)	6.2
AUC_{6-12h} (h*pg/mL)[2]	MYL-1601D vs. NovoLog®	68/68	75.60	(61.38; 92.16)	68.8
	MYL-1601D vs. NovoRapid®	67/67	81.37	(62.70; 105.75)	87.1
	NovoLog® vs. NovoRapid®	66/66	107.18	(83.81; 137.45)	82.1
$AUC_{0-\infty}$ (h*pg/mL)[1]	MYL-1601D vs. NovoLog®	68/68	101.87	(100.06; 103.72)	6.3
	MYL-1601D vs. NovoRapid®	67/67	101.74	(99.98; 103.54)	6.1
	NovoLog® vs. NovoRapid®	66/66	99.71	(98.04; 101.41)	5.8

[1] ANOVA; [2] Fieller's Theorem, [3] Geometric LS-mean/arithmetic mean ratio of treatments (test/reference), expressed in percent, [4] Two-sided 90% confidence intervals, [5] Intra-subject CV%

2.4.3. Pharmacodynamics

Mechanism of action

MYL-1601D is a rapid-acting human insulin analogue formulated as a solution for injection available in multi-dose vials and pre-filled pens.

The active substance in MYL-1601D 100 U/mL solution for injection is insulin aspart, a rapid-acting insulin analogue, administered subcutaneously by injection in the abdominal wall, the thigh, the upper arm, the deltoid region or the gluteal region. The blood glucose lowering effect of insulin aspart is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver. Insulin Aspart is also used for intravenous administration by physicians or other healthcare staff as applicable.

Primary and Secondary pharmacology

An euglycaemic clamp study was conducted in order to demonstrate similarity between PD of MYL-1601 and NovoRapid and NovoLog, respectively (Study MYL-1601D-1001). PD endpoints were obtained from glucose infusion rates measured by the ClampArt device over a period of 12 hours following an s.c. injection of 0.2 U/kg of MYL-1601D or NovoRapid or NovoLog. During the entire glucose clamp, the device measured blood glucose continuously, and the respective GIR was recorded every minute.

Primary PD Endpoints:

- $AUC_{GIR,0\text{-}last}$, area under the glucose infusion rate curve from 0 hours until the end of clamp
- GIR_{max}, maximum glucose infusion rate

Secondary PD Endpoints:

- $AUC_{GIR,0\text{-}4h}$, area under the glucose infusion rate curve from 0 to 4 hours
- $AUC_{GIR,0\text{-}6h}$, area under the glucose infusion rate curve from 0 to 6 hours
- $AUC_{GIR,6\text{-}last}$, area under the glucose infusion rate curve from 6 hours until the end of clamp
- $t_{max.GIR}$, time to maximum glucose infusion rate
- $t_{GIR,50\%\text{-}early}$, time to half-maximum glucose infusion rate before GIR_{max}
- $t_{GIR,50\%\text{-}late}$, time to half-maximum glucose infusion rate after GIR_{max} (indicator of end of duration of action)
- Onset of action, time from trial product administration until blood glucose concentration has decreased at least 5 mg/dL from baseline, where baseline is defined as the mean of blood glucose levels from -6, -4, and -2 minutes before trial product administration as measured by ClampArt
- Duration of action, as defined by difference between $t_{GIR,50\%\text{-}late}$ and onset of action (as defined above).

Standard statistical PK methods were applied in the analysis of the PD data. In the comparison with NovoLog, 90% CI was used (and were also used for the submission to the FDA), whereas in the comparison with NovoRapid 95% CI was used (for the EMA submission) in order to demonstrate similar pharmacodynamics of MYL-1601D and NovoLog and NovoRapid.

Furthermore, two sets of sensitivity analyses were conducted: i) where data from all subjects including low responders were used, and ii) where profiles with predefined C-peptide criteria were excluded.

Pharmacodynamic results

Figure 2 shows the overlaid mean smoothed GIR profiles of the three insulin aspart products, and Table 5 displays the results of the statistical comparisons for the PD endpoints.

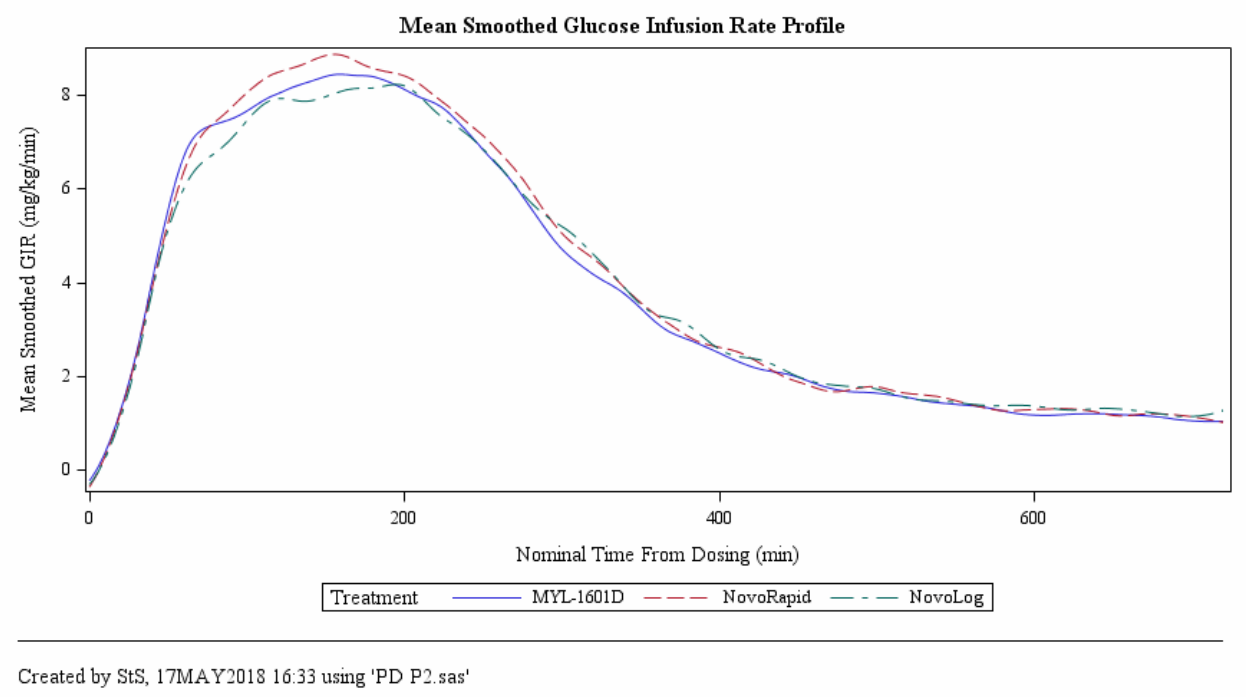
The 90% and 95% confidence intervals of the primary PD endpoints ($AUC_{GIR,0h\text{-}last}$ and GIR_{max}) lie within the prespecified range. The results from the sensitivity analysis, in which 2 subjects meeting the C-peptide criteria were excluded, showed similar estimates and CI as the main analysis.

In accordance with the lower insulin exposure of MYL-1601D at the end of the clamp (see PK section), the glucose infusion rate at the end of the clamp (from 6 hours onwards) are lower for MYL-1601D than Novolog,

but the 90% CI does not exceed the prespecified limits (80%-125%). No differences in glucose infusion rate between MYL-1601D and NovoRapid at the end of the clamp were observed.

The secondary end-points: time to maximum glucose infusion rate, time until 50% of $GIR_{max(early)}$, time until 50% of $GIR_{max(late)}$, onset of action and duration of action did not differ substantially between MYL-1601D, NovoRapid and NovoLog.

Figure 3: Mean Smoothed Glucose Infusion Rate Profile



Source: EOT Figure 14.2.4.4, MYL-1601D-1001 CSR

Table 2: Treatment comparisons for pharmacodynamic endpoints (PPP for PD)

Parameter	Comparison	N	Ratio %[3]	90% CI[4] 95% CI[5]
$AUC_{GIR,0h-last}$ (mg/kg)[1]	**MYL-1601D vs. NovoLog®**	**67/67**	**99.93**	**(95.74; 104.30)[4]**
	MYL-1601D vs. NovoRapid®	**67/67**	**96.42**	**(91.17; 101.98)[5]**
	NovoLog® vs. NovoRapid®	65/65	96.10	(92.37; 99.99)[4]
GIR_{max}(mg/kg/min)[1]	**MYL-1601D vs. NovoLog®**	**67/67**	**100.12**	**(94.46; 106.12)[4]**
	MYL-1601D vs. NovoRapid®	**67/67**	**95.10**	**(89.37; 101.19)[5]**
	NovoLog® vs. NovoRapid®	65/65	94.41	(89.38; 99.74)[4]
$AUC_{GIR,0-4h}$ (mg/kg)[2]	MYL-1601D vs. NovoLog®	67/67	103.31	(99.27; 107.53)[4]
	MYL-1601D vs. NovoRapid®	67/67	97.69	(93.60; 101.95)[5]

Parameter	Comparison	N	Ratio %[3]	90% CI[4] 95% CI[5]
	NovoLog® vs. NovoRapid®	65/65	94.11	(90.37; 97.99)[4]
$AUC_{GIR,0\text{-}6h}$ (mg/kg)[2]	MYL-1601D vs. NovoLog®	67/67	101.37	(97.88; 104.98)[4]
	MYL-1601D vs. NovoRapid®	67/67	96.37	(92.42; 100.49)[5]
	NovoLog® vs. NovoRapid®	65/65	94.81	(91.46; 98.27)[4]
$AUC_{GIR,6h\text{-}last}$ (mg/kg)[2]	MYL-1601D vs. NovoLog®	67/67	93.33	(88.79; 98.09)[4]
	MYL-1601D vs. NovoRapid®	67/67	94.77	(87.27; 102.87)[5]
	NovoLog® vs. NovoRapid®	65/65	101.05	(95.75; 106.64) [4]

[1] ANOVA; [2] Fieller's Theorem, [3] Geometric LS-mean/arithmetic mean ratio of treatments (test/reference), expressed in percent, [4] Two-sided 90% confidence intervals, [5] Two-sided 95% confidence intervals

2.4.4. Discussion on clinical pharmacology

Methodology

A single dose euglycaemic clamp study (MYL-1601D-1001) investigated the equivalence in pharmacokinetics between MYL-1601D and NovoRapid (EU-approved) and NovoLog (US-Licensed). The study was conducted in healthy men and women, and no studies in the target population were conducted. Clamp studies in healthy subjects are acceptable provided the evidence is presented that during the clamp period, the endogenous insulin secretion was completely supressed. Indeed, the MAH followed the C-peptide levels during the study.

The study was considered well conducted and the comparison between treatment groups are considered adequate.

Pharmacokinetics

The 90% confidence interval of the primary PK end-points ($AUC_{(0\text{-}12h)}$ and C_{max}) were within the predefined limits (80%-125%) for the comparison with NovoLog (US-licensed) as well as NovoRapid (EU-approved). Thus, similar pharmacokinetics between MYL-1601D, NovoLog and NovoRapid were documented.

At the end of the clamp, high variability was observed, and AUC was lower for MYL-1601D than Novorapid and NovoLog. This finding does not alone preclude biosimilarity.

Overall, the study was well-conducted, and similar pharmacokinetics of MYL-1601D, NovoLog and NovoRapid are considered documented

Pharmacodynamics

In the euglycaemic clamp study, pharmacodynamics were also investigated. Two primary pharmacodynamic endpoints (area under the glucose infusion rate curve from 0 hours until the end of clamp and the maximum glucose infusion rate), and other relevant secondary endpoints were investigated. The 95% confidence interval of the primary PD endpoints lie within the predefined range (80%-125%) for the comparison with NovoRapid, which is in accordance with the EMA guideline. For the comparison with NovoLog, the 90%

confidence interval was used due to the FDA requirements and was within the predefined range (80%-125%).

Overall, similar pharmacodynamic profiles have been demonstrated of MYL-1601D, NovoLog and NovoRapid.

The totality of clinical PK/PD data support biosimilarity between MYL-1601D and NovoLog (US-licensed) and NovoRapid (EU-approved).

2.4.5. Conclusions on clinical pharmacology

The available PK/PD data support biosimilarity between MYL-1601D and NovoRapid (EU approved) and NovoLog (US licenced). NovoLog is representative of the EU reference medicinal product (NovoRapid).

2.5. Clinical efficacy

Mylan insulin aspart, MYL-1601D is being developed by Mylan as a similar biological medicinal product to US-licensed NovoLog® (NovoLog) and EU-approved NovoRapid® (NovoRapid) for use in the same indications as approved in each region. In accordance with EMA Guidelines (EMEA/CHMP/BMWP/42832/2005 Rev1 and EMEA/CHMP/BMWP/32775/2005_Rev. 1), the development
program has aimed to establish similarity of MYL-1601D to the reference medicinal product (NovoRapid®) in terms of physicochemical characteristics, biological activity, pharmacokinetics (PK), pharmacodynamics and safety and efficacy, including immunogenicity.

2.5.1. Dose response study(ies)

Not applicable.

2.5.2. Main studies

The clinical study program for MYL-1601D includes the two studies summarized in table 2 below.
Dose-response studies are not applicable for this biosimilar application.

The methodology is described separately for each study below. Efficacy results are provided for the phase 3 study (MYL-1601D-3001) only.

Table 3 MYL-1601D and NovoLog® and NovoRapid® Used Across Studies

Clinical Studies	Comparator	Classification of the study	Purpose of Study	Status
MYL-1601D-1001: Phase 1 PK / PD equivalence; euglycemic clamp study; normal healthy volunteers (NHV) (Germany)	NovoLog® and NovoRapid®	Pivotal	To demonstrate PK and PD equivalence of MYL-1601D with US-Licensed NovoLog® and EU-approved NovoRapid®	Completed
MYL-1601D-3001: Phase 3 safety, immunogenicity and efficacy study in T1DM (US)	NovoLog®	Supportive	To compare the safety, immunogenicity and efficacy of MYL-1601D with NovoLog®	Completed

Abbreviations: NHV, normal healthy volunteers; PD- pharmacodynamic(s); PK- pharmacokinetic(s); T1DM - type 1 diabetes mellitus.
NovoLog® - sourced from the US and NovoRapid® sourced from the EU.

Study MYL-1601D-1001

Methods

71 healthy subjects aged 18 to 65 years, body mass index between 18.5 and 29.0 kg/m² and fasting plasma glucose ≤ 100 mg/dL were exposed to IMP (70 to MYL-1601D, 69 to NovoLog®, and 67 to NovoRapid®) with dosing of 0.2 U/kg body weight of each product. Safety endpoints included adverse events (AEs) including hypoglycemic episodes, hematology, biochemistry, urinalysis, Anti-insulin antibodies, physical examination, vital signs, electrocardiograms (ECGs), and local tolerability/reactions at the injection site.

Study MYL-1601D-3001

Methods

Study design

500 subjects, 18 to 65 years of age, with clinical diagnosis of type 1 diabetes mellitus for at least 6 months or more, with glycosylated hemoglobin (HbA_{1c}) 6.5-10%, on stable dose of once daily basal Lantus® or Toujeo® injection and multiple daily bolus NovoLog® or Humalog® injections for at least 3 months were planned to be included in the trial.

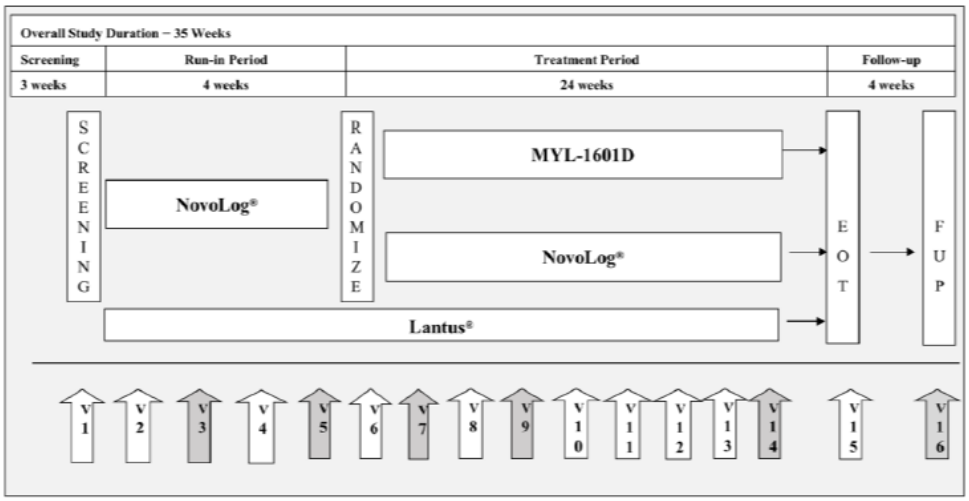
All patients were shifted to Lantus as basal insulin and FlexPen NovoLog in the screening phase and randomised to one of the following treatments in the treatment period:

- MYL-1601D Product (100 U/mL) or
- FlexPen NovoLog (100 U/mL)

taken at mealtime.

The study was a 24-week randomised, multicentre, open-label, parallel-group phase 3 study in subjects with T1DM comparing the safety and efficacy of MYL-1601D with NovoLog® (figure 9-1).

Figure 9–1 Study Diagram



Abbreviations: EOT = end of treatment; FUP = follow-up; V = visit

After a screening period of up to 3 weeks, all subjects were titrated on NovoLog during a 4-week run-in period and were shifted from their current basal insulin to study insulin Lantus. After the run-in period, subjects were randomized; 1 group received MYL-1601D, while the other group received NovoLog for 24 weeks. A follow-up visit, via telephone call, was scheduled 4 weeks after last dose of MYL-1601D or NovoLog.

A total of 500 subjects with T1DM were planned to be randomized in this study, to receive MYL-1601D or NovoLog with a randomization ratio of 1:1 (N = 250 subjects per treatment group).

Dosing with NovoLog during the run-in period and MYL-1601D or NovoLog during the treatment period was guided by SMBG-based glucose level assessments

Objectives:

The primary objective of study MYL-1601D-3001 was to assess treatment emergent antibody response (TEAR) at week 24 of MYL-1601D and compare to the reference product NovoLog. Additional measures of immunogenicity, efficacy and safety were assessed and compared between treatment groups. Of interest, the impact of ADAs on PD parameters were analysed.

Endpoints:

Primary Endpoint

The TEAR rate during the 24-week treatment period was the primary endpoint for this study.

A TEAR was defined as either one of the following:

1. Subjects who were ADA negative at baseline and became positive at any time point post-baseline.

2. Subjects who were ADA positive at baseline and demonstrated 4-fold increase in titer values at any time point post-baseline.

Secondary Endpoints

The secondary endpoints were change from baseline to Week 24.

Efficacy Endpoints

1. Change in HbA_{1c} from baseline
2. Change in FPG from baseline
3. Change in prandial, basal, and total daily insulin dose per unit body weight (U/kg) from baseline
4. Change in 7-point SMBG profile from baseline

Safety Endpoints

2. Incidence of positive antibody response and NAb
3. Impact of ADA on PD parameters, such as FPG, HbA_{1c}, and insulin dose
4. Change in hypoglycemia rate (30-day adjusted) from baseline and incidence of hypoglycemic events
5. Incidence of treatment emergent adverse events (TEAEs) and SAEs
6. Incidence of local reactions (includes injection site reaction), systemic reactions
7. Incidence of hypersensitivity and immune mediated AEs
8. Incidence of device related safety assessment

Sample size, randomization, blinding and statistical analyses:

The sample size estimation is based on primary objective – "to demonstrate that

immunogenicity as assessed by treatment emergent antibody response (TEAR) rate with MYL-1601D is equivalent to that of NovoLog® during 24-week treatment" as recommended by the US-FDA.

The randomisation procedure is adequately described. The randomisation was stratified by investigator and basal insulin dose and those were included as covariates in the statistical analyses. The design was open label, which could in principle have affected some of the endpoints. The primary endpoint was conducted with the ITT population.

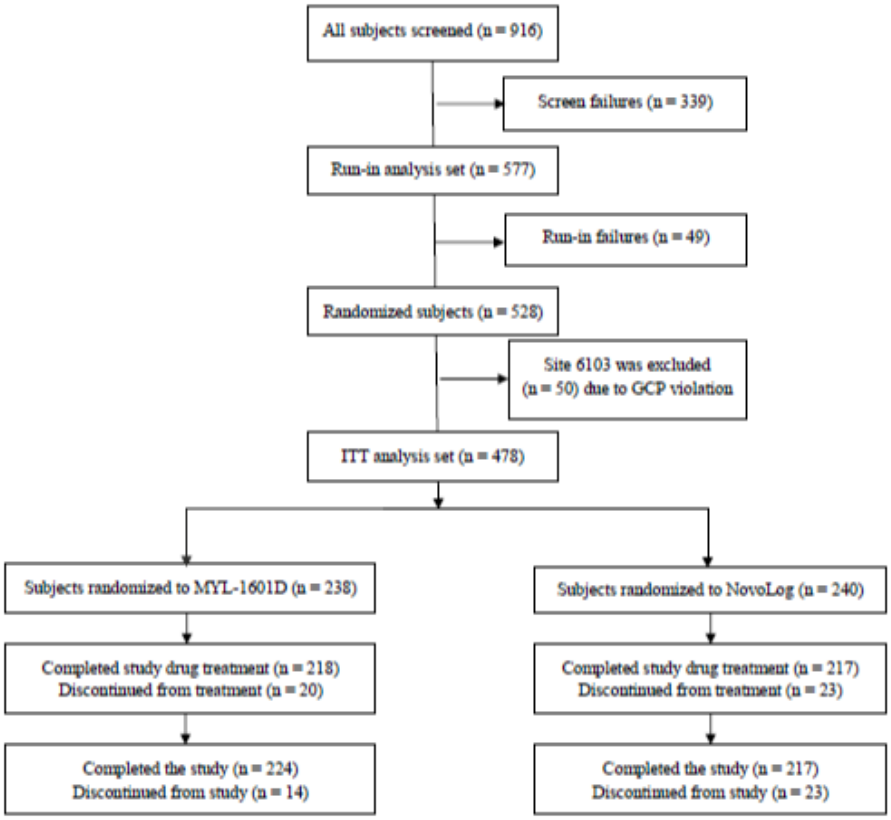
A site was excluded from the analysis due to GCP compliance issues. This was done before data lock.

Results

Participant flow

Participant flow is shown in Figure 10-1.

Figure 10–1 Subject Disposition Chart (ITT Analysis Set)



Source: Table 14.1.1.1, Listing 16.2.1.1
Abbreviations: n = number of subjects with data.

Details of subjects who discontinued from treatment due to AEs are provided in Section 12.3.1.3.

More than 90 % in both treatment groups completed treatment (Table 1). There were no apparent differences between groups in reasons for discontinuations. Patients who discontinued treatment were invited to stay in the study.

The Safety analysis set included subjects who took at least one dose of the study medication after randomization and without GCP violation.

Table 1: Summary of Subject Disposition (ITT Analysis Set)

	MYL-1601D	NovoLog	Total
Category	n (%)	n (%)	n (%[1])
ITT analysis set	238	240	478
Completed treatment	218 (91.6)	217 (90.4)	435 (91.0)
Discontinued from treatment	20 (8.4)	23 (9.6)	43 (9.0)
Subjects requested to discontinue treatment	13 (5.5)	8 (3.3)	21 (4.4)
Adverse event	3 (1.2)	2 (0.8)	5 (1.0)
Administrative decision by Sponsor	0	2 (0.8)	2 (0.4)
Investigator discretion	1 (0.4)	2 (0.8)	3 (0.6)
Lost to follow-up	3 (1.2)	9 (3.8)	11 (2.3)
Completed the study	224 (94.1)	217 (90.4)	441 (92.2)
Discontinued from study	14 (5.9)	23 (9.6)	38 (7.9)
Withdrew consent	7 (2.9)	8 (3.3)	15 (3.1)
Adverse event	1 (0.4)	2 (0.8)	3 (0.6)
Administrative decision by Sponsor	0	2 (0.8)	2 (0.4)
Investigator discretion	1 (0.4)	2 (0.8)	3 (0.6)
Lost to follow-up	4 (1.6)	9 (3.8)	13 (2.7)
Other	1 (0.4)	0	1 (0.2)

Source: Listing 16.2.1.1, MYL-1601D-3001 CSR

Note: n = number of subjects with data.

1. Percentages for subjects completing treatment and completing the study were calculated from the number of subjects in the ITT analysis set.

Baseline data

Baseline data are shown in Table 11-4. Baseline characteristics were comparable between treatment groups. Only two patients had a prior history of treatment with immunosuppressants (1 methotrexate, 1 other), both in the NovoLog treatment group. There were no apparent differences seen with the medical history between the treatment groups. There were no differences in treatment compliance.

Table 11-4 Summary of Baseline Characteristics (ITT Analysis Set)

Variable	MYL-1601D (N = 238) n (%)	NovoLog (N = 240) n (%)	Total (N = 478) n (%)
Duration of diabetes (years)			
n	238	240	478
Mean (standard deviation)	22.3 (13.71)	21.3 (12.91)	21.8 (13.31)
Median (Q1, Q3)	20.0 (11.0, 32.0)	20.0 (11.0, 29.5)	20.0 (11.0, 30.0)
Minimum, maximum	1, 58	1, 58	1, 58
Time of basal dose, n (%)			
Morning	84 (35.3)	91 (37.9)	175 (36.6)
Evening	154 (64.7)	149 (62.1)	303 (63.4)
Baseline fasting plasma glucose (mmol/L)			
n	237	237	474
Mean (standard deviation)	8.944 (3.9321)	9.218 (3.8593)	9.081 (3.8941)
Median (Q1, Q3)	8.500 (6.430, 10.780)	8.770 (6.500, 11.650)	8.640 (6.440, 11.250)
Minimum, maximum	2.05, 24.34	1.95, 25.04	1.95, 25.04
Baseline HbA1c (%)			
n	238	240	478
Mean (standard deviation)	7.85 (0.868)	7.81 (0.784)	7.83 (0.827)
Median	7.80 (7.20, 8.50)	7.70 (7.30, 8.25)	7.70 (7.20, 8.40)
Minimum, maximum	5.7, 10.5	6.1, 10.3	5.7, 10.5
Insulin use prior to screening, n (%)			
Yes	238 (100)	240 (100)	478 (100)
No	0	0	0

Source: Table 14.1.3.1
Abbreviations: HbA1c = glycosylated hemoglobin; ITT = Intent-to-treat; N = number of subjects in the population; n = number of subjects with data; Q1 = 25th percentile; Q3 = 75th percentile.
Note: Percentages were based on N.

Numbers analysed

The ITT set included 478 patients, and the PP analysis set 432 patients.

Outcomes and estimation

Primary endpoint

The TEAR rate for MYL-1601D was 59/238 (24.9%), and the TEAR rate for NovoLog was 67/240 (27.8%) with a treatment difference of -2.86% (90% CI -9.71; 3.99) which is in the prespecified martin of ±12% (Table 11.8).

Table 11-8 Summary of Treatment Emergent Antibody Response Rate at Week 24 with Multiple Imputation (ITT Analysis Set)

Outcome	MYL-1601D (N = 238) n (%)	NovoLog (N = 240) n (%)	Treatment Difference (SE)	90% CI	± Margin (%)
TEAR responders	59 (24.9)	67 (27.8)	-2.86 (4.16)	-9.71, 3.99	12.0
TEAR non-responders	179 (75.1)	173 (72.2)			

Source: Table 14.2.1.1
Abbreviations: ADA = anti-drug antibody; CCMV = complete-case missing values; CI = confidence interval; ITT = Intent-to-treat; N = number of subjects in population; n = number of subjects calculated from the response rate; SE = standard error; TEAR = treatment emergent antibody response.
TEAR responder was defined as either 1 of the following:

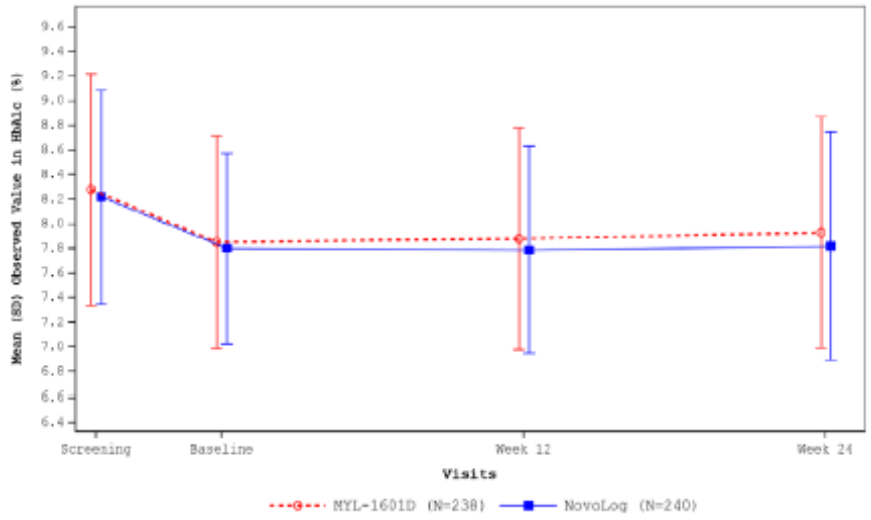
1 Subjects who were ADA negative at baseline and became positive at any time point post-baseline.
2 Subjects who were ADA positive at baseline and demonstrated a 4-fold increase in titer values at any time point post-baseline.

The 90% CI of the treatment difference (MYL-1601D minus NovoLog) in the TEAR rate was established using the Wald confidence limit method. The CCMV method was used.
The margin was calculated based on the sample size and TEAR (observed and imputed) rate from the NovoLog group.

Secondary endpoints

Analysis of secondary endpoints showed stable HbA1c, fasting plasma glucose, prandial and basal insulin dose during 24 weeks of treatment (Figure 11-3, Table 11-11, Figure 11-4, Table 11-13, Figure 11-5 and Table 11-14, and Figure 14.2.6.6).

Figure 11–3 Means and Standard Deviations for Observed HbA1c (%) Values Over Time by Treatment (ITT Analysis Set)



Source: Figure 14.2.2.3
Abbreviations: HbA1c = glycosylated hemoglobin; ITT = Intent-to-treat; N = number of subjects in population; SD = standard deviation.
Means and standard deviations are from descriptive statistics procedure.

Table 11-11 Summary of HbA1c (%) Observed Values and Change from Baseline by Visit (ITT Analysis Set)

Treatment Variable	Statistics	Baseline	Week 12	Week 24
Observed	n	229	230	226
	Mean (SD)	7.85 (0.864)	7.88 (0.901)	7.93 (0.941)
	Min, median, max	5.7, 7.80, 10.5	5.8, 7.80, 11.6	5.8, 7.90, 11.4
Change	n	NA	221	217
MYL-1601D	Mean (SD)	NA	0.04 (0.615)	0.10 (0.735)
(N = 238)	LS means (SE)	NA	0.05 (0.039)	0.11 (0.048)
	Min, median, max	NA	-2.0, 0.00, 3.7	-2.3, 0.10, 2.6
	P-value [a]	NA	0.2849	0.0424
Observed	n	235	224	216
	Mean (SD)	7.80 (0.774)	7.79 (0.843)	7.82 (0.925)
	Min, median, max	6.1, 7.70, 10.3	6.1, 7.70, 10.5	5.9, 7.70, 11.3
Change	n	NA	221	211
NovoLog	Mean (SD)	NA	-0.01 (0.547)	0.04 (0.723)
(N = 240)	LS means (SE)	NA	0.00 (0.039)	0.04 (0.048)
	Min, median, max	NA	-1.7, 0.00, 1.7	-1.9, 0.00, 3.1
	P-value [a]	NA	0.7309	0.4028
MYL-1601D – NovoLog	Treatment difference	NA	0.05	0.07
	95% CI of difference	NA	(-0.05, 0.16)	(-0.06, 0.20)
	P-value [b]	NA	0.3336	0.3165

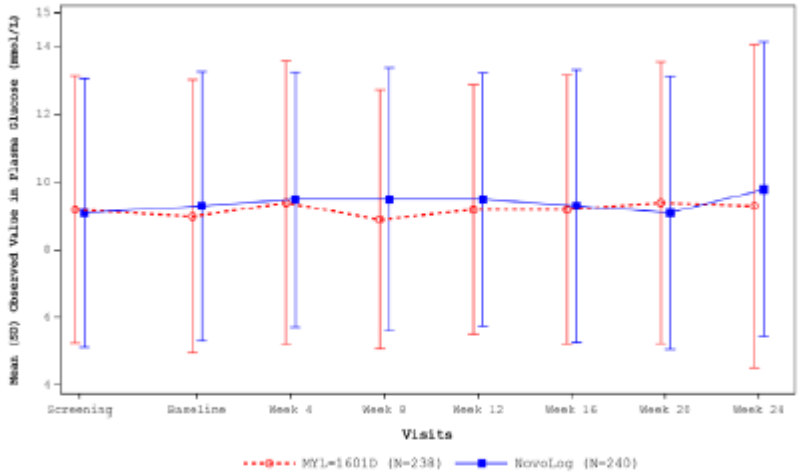
Source: Table 14.2.2.1.1

Abbreviations: CI = confidence interval HbA1c = glycosylated hemoglobin; ITT = Intent-to-treat; LS means = least squares means; min = minimum; max = maximum; N = number of subjects in population; n = number of subjects with data at the time point; NA = not applicable; SD = standard deviation; SE = standard error.

a *P*-value based on paired t-test.

b *P*-value based on a mixed model with repeated measures with the change from baseline value as a dependent variable and treatment group, visit (Weeks 12 and 24), the treatment group by visit interaction, basal insulin dose time, and pooled sites as fixed effects, as well as the baseline value as a covariate.

Figure 11–4 Means and Standard Deviations for Observed Plasma Glucose (mmol/L) Values Over Time by Treatment (ITT Analysis Set)



Source: Figure 14.2.3.4
Abbreviation: ITT = Intent-to-treat; N = number of subjects in population; SD = standard deviation.
Means and standard deviations are from descriptive statistics procedure.

Table 11-13 Summary of Plasma Glucose (mmol/L) Observed Values and Change from Baseline by Visit (ITT Analysis Set)

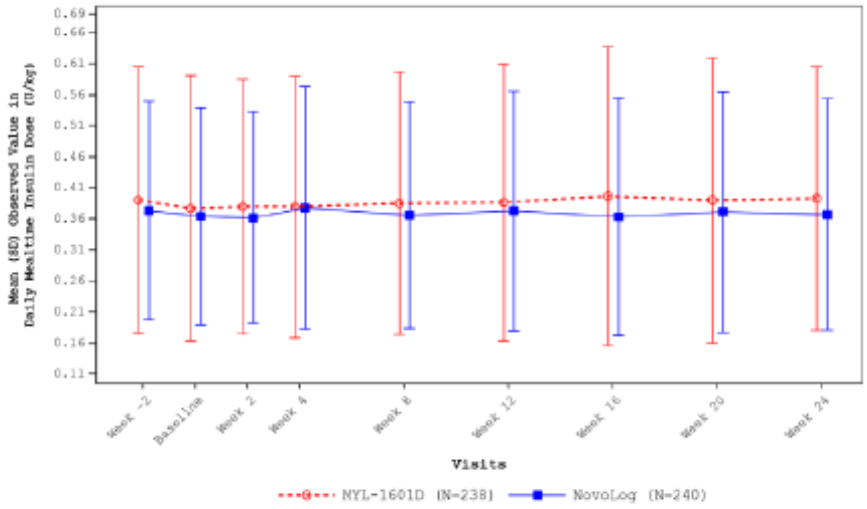
Treatment Variable	Statistics	Baseline	Week 12	Week 24
Observed	n	230	227	220
	Mean (SD)	9.022 (4.0313)	9.228 (3.6856)	9.326 (4.7777)
	Min, median, max	2.05, 8.515, 24.34	1.88, 8.980, 19.95	2.02, 8.490, 31.45
Change	n	NA	220	214
MYL-1601D	Mean (SD)	NA	0.149 (5.1749)	0.400 (5.8162)
(N = 238)	LS means (SE)	NA	0.174 (0.2490)	0.356 (0.3093)
	Min, median, max	NA	-15.45, 0.020, 14.06	-13.65, -0.075, 27.01
	P-value [a]	NA	0.6698	0.3155
Observed	n	236	218	211
	Mean (SD)	9.282 (3.9844)	9.527 (3.7532)	9.787 (4.3600)
	Min, median, max	1.95, 8.765, 25.04	1.78, 9.170, 19.56	2.94, 9.070, 32.84
Change	n	NA	215	208
NovoLog	Mean (SD)	NA	0.365 (4.5402)	0.599 (5.1553)
(N = 240)	LS means (SE)	NA	0.503 (0.2510)	0.717 (0.3124)
	Min, median, max	NA	-14.84, 0.100, 12.13	-14.29, 0.320, 16.19
	P-value [a]	NA	0.2392	0.0955
MYL-1601D –	Treatment difference	NA	-0.329	-0.361
NovoLog	95% CI of difference	NA	(-1.015, 0.357)	(-1.218, 0.496)
	P-value [b]	NA	0.3459	0.4084

Source: Table 14.2.3.1
Abbreviations: CI = confidence interval; ITT = Intent-to-treat; LS means = least squares means; min = minimum; max = maximum; N = number of subjects in population; n = number of subjects with data at the time point; NA = not applicable; SD = standard deviation; SE = standard error.

a *P*-value based on paired t-test.

b *P*-value based on a mixed model with repeated measures with the change from baseline value as a dependent variable and treatment group, visit (Weeks 12 and 24), the treatment group by visit interaction, basal insulin dose time, and pooled sites as fixed effects, as well as the baseline value as a covariate.

Figure 11–5 Means and Standard Deviations for Observed Daily Mealtime Insulin Dose (U/kg) Values Over Time by Treatment (ITT Analysis Set)



Source: Figure 14.2.4.3
Abbreviation: ITT = Intent-to-treat; N = number of subjects in population; SD = standard deviation.
Means and standard deviations are from descriptive statistics procedure.

Table 11-14 Analysis of Daily Mealtime Insulin Dose (U/kg) Baseline Values and Change from Baseline (ITT Analysis Set)

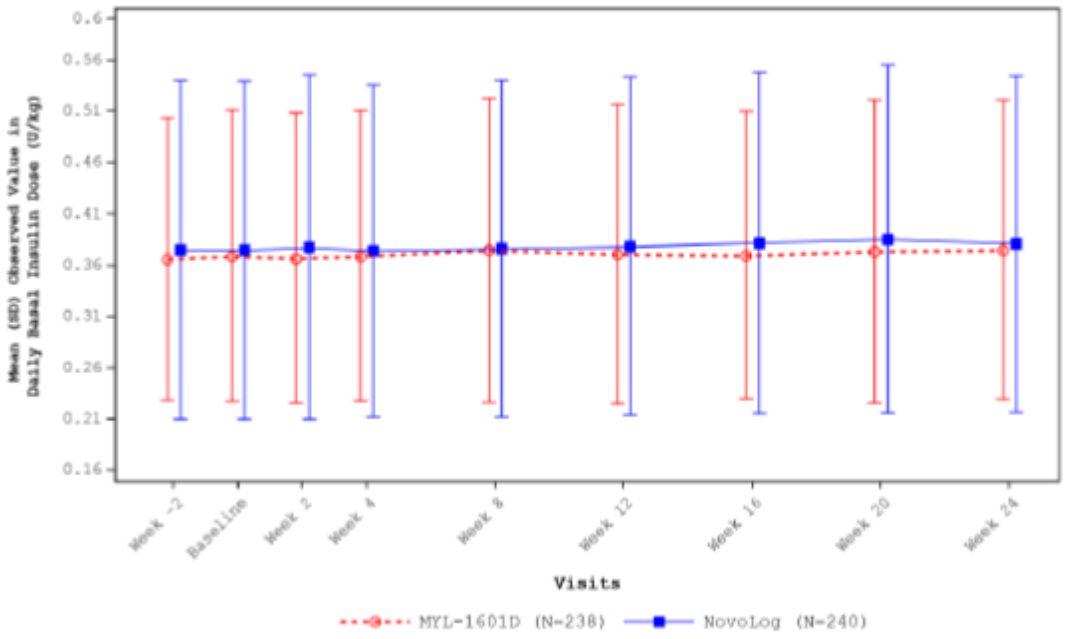
Treatment Variable	Statistics	Baseline Values	Change from Baseline						
			Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
MYL-1601D (N = 238)	n	221	211	210	199	204	194	196	193
	Mean (SD)	0.3776 (0.21342)	0.0045 (0.10904)	0.0038 (0.12122)	0.0011 (0.12586)	0.0035 (0.13387)	0.0085 (0.13582)	0.0087 (0.14296)	0.0079 (0.14608)
	LS means (SE)	NA	0.0055 (0.00629)	0.0048 (0.00743)	0.0033 (0.00708)	0.0082 (0.00814)	0.0131 (0.00810)	0.0117 (0.00833)	0.0102 (0.00817)
	Min, median, max	0.063, 0.3380, 1.545	-1.094, 0.0090, 0.410	-0.871, 0.0000, 0.531	-1.053, 0.0040, 0.396	-1.096, 0.0005, 0.438	-1.077, 0.0045, 0.430	-1.119, 0.0000, 0.517	-1.201, 0.0020, 0.483
	P-value [a]	NA	0.5455	0.6493	0.9015	0.7088	0.3859	0.3964	0.4542
NovoLog (N = 240)	n	220	208	205	202	197	191	188	190
	Mean (SD)	0.3649 (0.17540)	0.0010 (0.07521)	0.0073 (0.09746)	0.0004 (0.08572)	0.0060 (0.10461)	-0.0061 (0.10073)	-0.0025 (0.10251)	-0.0008 (0.09731)
	LS means (SE)	NA	-0.0017 (0.00632)	0.0057 (0.00750)	-0.0018 (0.00710)	0.0050 (0.00824)	-0.0062 (0.00817)	-0.0031 (0.00843)	-0.0001 (0.00824)
	Min, median, max	0.072, 0.3365, 1.010	-0.531, 0.0025, 0.276	-0.511, 0.0030, 0.503	-0.608, 0.0000, 0.305	-0.553, 0.0030, 0.358	-0.473, -0.0080, 0.365	-0.517, -0.0020, 0.473	-0.388, -0.0050, 0.361
	P-value [a]	NA	0.8546	0.2830	0.9510	0.4226	0.4061	0.7342	0.9099
MYL-1601D – NovoLog	Treatment difference	NA	0.0072	-0.0009	0.0051	0.0032	0.0193	0.0148	0.0103
	95% CI of difference	NA	(-0.0098, 0.0242)	(-0.0212, 0.0194)	(-0.0142, 0.0244)	(-0.0192, 0.0256)	(-0.0029, 0.0415)	(-0.0081, 0.0377)	(-0.0121, 0.0327)
	P-value [b]	NA	0.4048	0.9313	0.6028	0.7790	0.0885	0.2046	0.3656

Source: Table 14.2.4.1
Abbreviations: CI = confidence interval; ITT = Intent-to-treat; LS means = least squares means; min = minimum; max = maximum; N = number of subjects in population; n = number of subjects with data at the time point; SD = standard deviation; NA = not applicable; SE = standard error.

a *P*-value based on paired t-test.

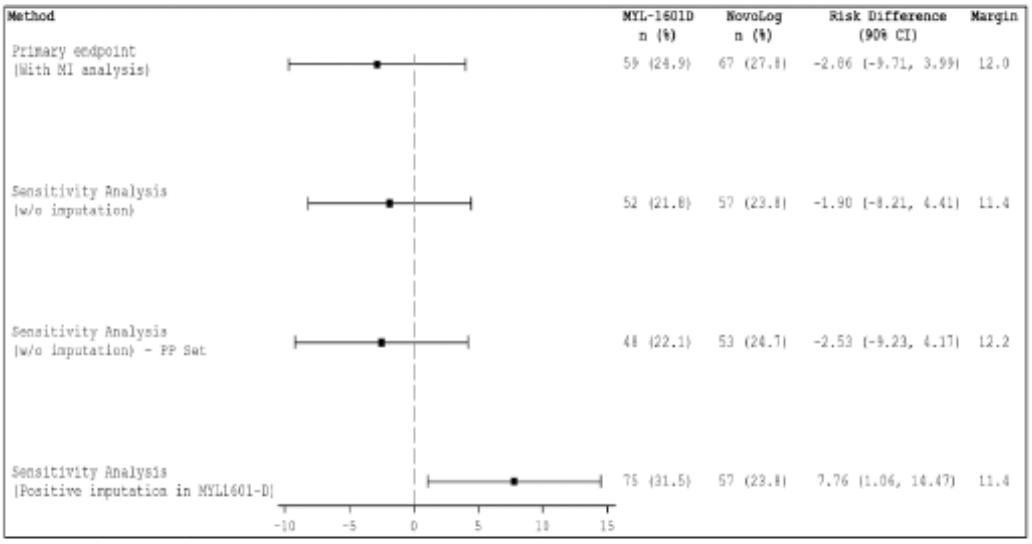
b *P*-value based on a mixed model with repeated measures with the change from baseline value as a dependent variable and treatment group, visit (Weeks 2, 4, 8, 12, 16, 20, and 24), the treatment group by visit interaction, basal insulin dose time, and pooled sites as fixed effects, as well as the baseline value as a covariate.

Figure 14.2.6.6
Means and SD for the Observed Daily Basal Insulin Dose (U/kg) Values over Time and Treatment
Intent to Treat Analysis Set



The sensitivity analysis pointed in the same direction as the analysis of the primary endpoint, with an exception of the positive imputation showing a statistically significant higher TEAR rate (Figure 2). This represents an extreme situation, and as the majority of the scenarios (95.4%) points towards no difference between groups, the sensitivity analyses are considered to support the primary analysis.

Figure 2 Forest Plot for Primary and Sensitivity Analyses, Study MYL-1601D-3001



Source: Figure 14.2.1.1, MYL-1601D 3001 CSR

Abbreviations: ADA = anti-drug antibody; CI = confidence interval; ITT = Intent-to-treat; n = number of subjects calculated from the response rate; MI = multiple imputation; PP = Per-protocol; TEAR = treatment emergent antibody response; w/o = without.

TEAR responder was defined as either 1 of the following:

3 Subjects who were ADA negative at baseline and became positive at any time point post-baseline.

4 Subjects who were ADA positive at baseline and demonstrated a 4-fold increase in titer values at any time point post-baseline.

The 90% CI of the treatment difference (MYL-1601D minus NovoLog) in the TEAR rate was established using the Wald confidence limit method. The margin was calculated based on the sample size and TEAR rate from the NovoLog® group.

Summary of main study(ies)

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the biosimilarity assessment (see later sections).

Table 1. Summary of Efficacy for trial myl-1601d-3001

Title: A Randomized, Multicenter, Open-Label, Parallel-Group Clinical Study Comparing the Safety and Efficacy of MYL-1601D with NovoLog® in Type 1 Diabetes Mellitus Patients		
Study	MYL-1601D-3001	
Design	Open-label, randomized, multicenter, parallel-group active controlled, 2 arm study	
	Screening Duration of Run-in phase: Duration of main phase: Safety Follow-up:	Up to 4 weeks 4 weeks 24 weeks 4 weeks
Hypothesis	Equivalence	

Treatments groups	MYL-1601D		MYL-1601D Product (100 U/mL), 263 subjects randomized, 24 weeks
	NovoLog®		FlexPen® NovoLog® (100 U/mL), 265 subjects randomized, 24 weeks
Endpoints and definitions	Primary endpoint	Treatment emergent antibody response (TEAR)	Subjects who were ADA negative at baseline and became positive at any time point post-baseline. Subjects who are ADA positive at baseline and demonstrate 4-fold increase in titer values at any timepoint post baseline
	Secondary endpoint	HbA1c	Change in HbA1c from baseline
	Secondary endpoint	FPG	Change in fasting plasma glucose (FPG) from baseline
	Secondary endpoint	Prandial insulin dose	Change in prandial insulin dose per unit body weight from baseline
	Secondary endpoint	Basal insulin dose	Change in basal insulin dose per unit body weight from baseline
	Secondary endpoint	Total daily insulin dose	Change in total daily insulin dose per unit body weight from baseline
	Secondary endpoint	Daily mean of SMBG	Change in 7-point SMBG profile from baseline
	Secondary endpoint	Safety parameters	Occurrence of local reactions, systemic reactions, Incidence and rate of hypoglycemic events and other adverse events
	Secondary endpoint	Incidence of positive ADA	Incidence of positive antibody response
Database lock	3rd March, 2020		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (ITT) Primary endpoint was analysed at 24 Weeks The Intent-to-treat (ITT) analysis set included all randomized subjects (including subjects who received incorrect treatment, did not complete the study, or did not comply with the protocol or used prohibited medication, or who were randomized but did not take any study treatment). The subjects who were in the site with a GCP violation were excluded from ITT analysis set.		
Descriptive statistics and estimate variability	Treatment group	MYL-1601D	NovoLog®
	Number of subjects	238	240

	Proportion of subjects met with TEAR criteria Number of subjects (%)	59 (24.9)	67 (27.8)
	HbA1c (%) (SE) LSmean change from baseline at week 24	0.11 (0.048)	0.04 (0.048)
	FPG (mmol/L) (SE) LSmean change from baseline at week 24	0.356 (0.3093)	0.717 (0.3124)
	Prandial insulin dose (U/kg) (SE) LSmean change from baseline at week 24	0.0102 (0.00817)	-0.0001 (0.00824)
	Basal insulin dose (U/kg) (SE) LSmean change from baseline at week 24	0.0078 (0.00388)	0.0029 (0.00392)
	Total daily insulin dose (U/kg) (SE) LSmean change from baseline at week 24	0.0210 (0.00926)	0.0038 (0.00936)
	Daily mean of SMBG (mmol/L) (SE) LSmean change from baseline at week 24	0.1958 (0.11366)	0.1110 (0.11464)
	Incidence of positive ADA Number of subjects (%)	174 (73.1)	166 (69.2)
Effect estimate per comparison	Primary Endpoint: TEAR	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference (SE)	-2.86 (4.16)
		90% CI of treatment difference and equivalence margin	-9.71, 3.99 ±12.0
	Secondary Endpoint: HbA1c	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	0.07

		95% CI of difference	-0.06, 0.20
		P-value	0.3165
	Secondary Endpoint: FPG	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	-0.361
		95% CI of difference	(-1.218, 0.496)
		P-value	0.4084
	Secondary Endpoint: Prandial insulin dose	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	0.0103
		95% CI of difference	(-0.0121, 0.0327)
		P-value	0.3656
	Secondary Endpoint: Basal insulin dose	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	0.0049
		95% CI of difference	(-0.0058, 0.0156)
		P-value	0.3688
	Secondary Endpoint: Total daily insulin dose	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	0.0172
		95% CI of difference	(-0.0082, 0.0426)
		P-value	0.1833
	Secondary Endpoint: Daily mean of SMBG	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	0.0848
		95% CI of difference	(-0.2273, 0.3969)
		P-value	0.5936

	Secondary Incidence of positive ADA	Comparison groups: MYL-1601D vs Novolog®	MYL-1601D Product (100 U/mL) vs FlexPen® NovoLog® (100 U/mL)
		Treatment Difference	3.9
		90% CI of difference	(-2.87, 10.75)
		P-value	0.3416

Clinical studies in special populations

The majority of the patients were between 21 and 65 years old (N=441). In the youngest age group (<= 21 years) 25 patients were included. In the oldest age group (>= 65 years) 12 patients were included. Patients were evenly distributed between treatment groups within each age group.

There was a tendency towards a higher TEAR rate in the youngest age group, however the TEAR rate did not differ between treatment groups, neither in the youngest age group nor in the older age groups (Table 11-26).

Table 11-16 Analysis of Treatment Emergent Antibody Response Rate at Week 24 on Observed Cases by Age Group (ITT Analysis Set)

Sub Group Factor	Outcome	MYL-1601D (N = 238) n (%)	NovoLog (N = 240) n (%)	Total (N = 478) n (%)	Treatment Difference (SE)	90% CI
≤ 21	TEAR responders	4 (40.0)	5 (33.3)	9 (36.0)	6.67 (19.70)	-25.74, 39.07
	TEAR non-responders	5 (50.0)	7 (46.7)	12 (48.0)		
	Unconfirmed	1 (10.0)	3 (20.0)	4 (16.0)		
> 21 to < 65	TEAR responders	47 (21.2)	50 (22.8)	97 (22.0)	-1.66 (3.94)	-8.15, 4.83
	TEAR non-responders	153 (68.9)	146 (66.7)	299 (67.8)		
	Unconfirmed	22 (9.9)	23 (10.5)	45 (10.2)		
≥ 65	TEAR responders	1 (16.7)	2 (33.3)	3 (25.0)	-16.67 (24.53)	-57.02, 23.69
	TEAR non-responders	5 (83.3)	4 (66.7)	9 (75.0)		
	Unconfirmed	0	0	0		

Source: Table 14.2.1.7
Abbreviations: ADA = anti-drug antibody; CI = confidence interval; ITT = Intent-to-treat; N = number of subjects in population; n = number of subjects with data at the time point; SE = standard error; TEAR = treatment emergent antibody response.
TEAR responder was defined as either 1 of the following:

1 Subjects who were ADA negative at baseline and became positive at any time point post-baseline.
2 Subjects who were ADA positive at baseline and demonstrated a 4-fold increase in titer values at any time point post-baseline.

The 90% CI of the treatment difference (MYL-1601D minus NovoLog) in the TEAR rate was established using the Wald confidence limit method without imputation. Percentages were based on the number of subjects in the population in each subgroup.

Analysis of the TEAR rate by gender at Week 24 for the ITT analysis set is summarized in Table 11-17. Similar results were observed for both male and female subjects.

2.5.3. Discussion on clinical efficacy

The Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues (EMEA/CHMP/BMWP/32775/2005_Rev. 1) declares no anticipated need for specific efficacy studies, since endpoints (usually HbA_{1c}) used in such studies are not considered sensitive enough to detect potentially clinically relevant differences between two insulins.

The clinical study program for MYL-1601D includes two clinical studies, namely study MYL-1601D-1001 (including single-dosing of the three IMPs in 71 healthy subjects) and study MYL-1601D-3001.The efficacy evaluation is based on the MYL-1601D-3001 study due to the single-dose application of investigational medicinal products in study MYL-1601D-1001. The data from study MYL-1601D-3001 has been submitted with the day 121 response.

Design and conduct of clinical studies

In line with regulatory guidance, a 24-week study with the primary objective to demonstrate comparable immunogenicity has been conducted. The final CSR has been submitted with the Day 120 question responses.

A randomised, open-label, parallel-group phase 3 study in patients with type 1 diabetes was conducted. The primary objective of study MYL-1601D-3001 was to assess treatment emergent antibody response (TEAR) at week 24 of MYL-1601D and compare to the reference product NovoLog. Additional measures of immunogenicity, efficacy and safety were assessed and compared between treatment groups. Of interest, the impact of ADAs on PD parameters was analysed. The primary and secondary endpoints are supported and the definition of TEAR is agreed.

The open-label design is not expected to have an impact on the primary endpoint measuring immunogenicity. Some effect on other endpoints, including use of supplementary insulin, could in principle be affected as patients may be more alert if they know they are in the MYL-1601D arm. No major differences were noticed however.

The statistical methods are acceptable. A site was excluded from the analysis due to GCP compliance issues. This was done before data lock and is therefore acceptable. The primary endpoint was conducted with the ITT population, which is agreed.

More than 90 % in both treatment groups completed treatment. There were no apparent differences between groups in reasons for discontinuations. Patients who discontinued treatment were invited to stay in the study. This is endorsed.

Overall, the study appears to be well-conducted.

Efficacy data and additional analyses

Baseline characteristics were comparable between treatment groups. Only two patients had a prior history of treatment with immunosuppressants (1 methotrexate, 1 other), both in the NovoLog treatment group. There were no apparent differences seen with the medical history between the treatment groups and there were no differences in treatment compliance. Overall, the treatment groups are comparable.

The ITT set included 478 patients, and the PP analysis set 432 patients.

The primary endpoint, the TEAR rate, was 59/238 (24.9%) for MYL-1601D, and 67/240 (27.8%) for NovoLog with a treatment difference of -2.86% (90% CI -9.71; 3.99), which is in the prespecified margin of ±12%.

Analysis of the secondary endpoints showed stable HbA_{1c}, fasting plasma glucose, prandial and basal insulin dose during 24 weeks of treatment.

The Applicant has conducted several sensitivity analyses in order to address the missing data. The sensitivity analysis pointed in the same direction, with an exception of the positive imputation showing a statistically significant higher TEAR rate with MYL-1601D compared with NovoLog. It is agreed with the Applicant that this represents an extreme situation in which all the missing values is imputed as positive antibody response. As the majority of the scenarios (95.4%) points towards no difference between groups, the sensitivity analyses are considered to support the primary analysis.

The majority of the included patients were between 21 and 65 years old (N=441). In the youngest age group (<= 21 years) 25 patients were included. In the oldest age group (>= 65 years) 12 patients were included. Patients were evenly distributed between treatment groups within each age group. There was a tendency towards a higher TEAR rate in the youngest age group, however the TEAR rate did not differ between treatment groups, neither in the youngest age group nor in the older age groups.

In conclusion, based on the results of the primary and secondary endpoints and the sensitivity analyses, no differences in TEAR were observed between MYL-1601D and NovoLog, and no differences in effect parameters were observed.

2.5.4. Conclusions on the clinical efficacy

Overall, study MYL-1601D-3001 is considered well-conducted and no difference in TEAR and other efficacy endpoints were observed between MYL-1601D and the reference medicinal product.

2.6. Clinical safety

In accordance with EMA Guidelines (EMEA/CHMP/BMWP/42832/2005_Rev1 and EMEA/CHMP/BMWP/32775/2005_Rev. 1), the development program has aimed to establish similarity of MYL-1601D to the reference medicinal product (NovoRapid®) in terms of safety, including immunogenicity.

Patient exposure

The clinical study program for MYL-1601D includes the two studies summarized in table 2 below. Study MYL-1601D-1001 was an euglycemic clamp study in healthy volunteers, and study 3001 was a randomised, multicentre, open-label, parallel-group clinical study comparing the safety, immunogenicity, and efficacy of MYL-1601D with NovoLog® in Type 1 Diabetes Mellitus (T1DM) patients.

Table 4 MYL-1601D and NovoLog® and NovoRapid® Used Across Studies

Clinical Studies	Comparator	Classification of the study	Purpose of Study	Status
MYL-1601D-1001:	NovoLog® and NovoRapid®	Pivotal	To demonstrate PK and PD equivalence of MYL-1601D with US-Licensed NovoLog®	Completed

Clinical Studies	Comparator	Classification of the study	Purpose of Study	Status
Phase 1 PK / PD equivalence; euglycemic clamp study; normal healthy volunteers (NHV) (Germany)			and EU-approved NovoRapid®	
MYL-1601D-3001: Phase 3 safety, immunogenicity and efficacy study in T1DM (US)	NovoLog®	Supportive	To compare the safety, immunogenicity and efficacy of MYL-1601D with NovoLog®	Completed

Abbreviations: NHV, normal healthy volunteers; PD- pharmacodynamic(s); PK-pharmacokinetic(s); T1DM - type 1 diabetes mellitus.
NovoLog® - sourced from the US and NovoRapid® sourced from the EU.

The designs of the two clinical studies imply that safety evaluation should mainly be based on the MYL-1601D-3001 study. Data from study MYL-1601D-3001 have been submitted with the day 121 response, and the evaluation of safety is primarily based on this study. In the following, the evaluation of safety in study 3001 is provided first, and subsequently the evaluation of study 1001 is provided.

Study MYL-1601D-3001

The safety population from study 3001 consisted of 478 patients of which 238 had at least one dose of MYL-1601-D (Table 12-1). The baseline characteristics were similar between treatment groups. Around half of the patients were men and the majority were of white ethnicity. Mean diabetes duration was 21-22 years, and 34-35% had hypertension. Overall, the characteristics were well balanced between treatment groups, and the population included in study 3001 is adequately reflecting an adult type 1 diabetes population.

Table 12-1 Summary of Study Medication Exposure in Treatment Period (ITT Analysis Set)

	MYL-1601D (N = 238)	NovoLog (N = 240)	Total (N = 478)
Duration of exposure (days)			
n	238	240	478
Mean (standard deviation)	160.2 (32.34)	157.9 (34.87)	159.0 (33.62)
Median	168.0	168.0	168.0
Minimum, maximum	2, 203	1, 224	1, 224

Source: Table 14.1.6.1
Abbreviations: ITT = Intent-to-treat; N = number of subjects in the population.
Interruptions, compliance and dose changes were not taken into account for duration of exposure.
Exposure to study medication = date of last randomized study medication intake – date of first randomized study medication intake + 1.

Adverse events

The *proportion* of patients with treatment emergent adverse events was numerically higher for MYL-1601D (45.4%) compared with NovoLog (42.9%). The *number* of events were also higher with MYL-1601D (256) compared with NovoLog (231). Additionally, the percentage of patients with at least 1 severe adverse event or TEAE grade 3 or higher was numerically higher for MYL-1601D (9.2% and 9.2%) compared with NovoLog (6.3% and 5.8%). 3 patients treated with MYL-1601D discontinued treatment due to TEAE whereas 1 patient treated with NovoLog discontinued treatment due to TEAE. One patient died. This patient was in the NovoLog group.

The specific adverse events were similar between the treatment groups, besides hypoglycaemia, headache and diarrhoea that were more pronounced in the MYL-1601D group than NovoLog. 17 patients (7.1%) in the MYL-1601D group had a hypoglycaemic event whereas 10 patients (4.2%) in the NovoLog group had a hypoglycaemic event.

Overall, the number of subjects experiencing an adverse event, and the number of events were higher in the MYL-1601D group than in the NovoLog group. The difference is considered small (108 patients out of 238 vs 105 patients out of 240). The Applicant discusses that the higher frequency of adverse events can be attributed to the open label study design, which is agreed.

Table 12-3 Overall Summary of Adverse Events During Treatment Period (Safety Analysis Set)

Category	MYL-1601D (N = 238) n (%)/#events	NovoLog (N = 240) n (%)/#events	Total (N = 478) n (%)/#events
Number of subjects with at least 1 adverse event	108 (45.4)/256	103 (42.9)/231	211 (44.1)/487
Number of subjects with at least 1 TEAE	108 (45.4)/256	103 (42.9)/231	211 (44.1)/487
Number of subjects with at least 1 SAE	22 (9.2)/27	15 (6.3)/23	37 (7.7)/50
Number of subjects with at least 1 TEAE Grade 3 or higher	22 (9.2)/32	14 (5.8)/22	36 (7.5)/54
Number of subjects with at least 1 treatment related TEAE	18 (7.6)/24	10 (4.2)/13	28 (5.9)/37
Number of subjects who discontinued the study treatment due to a TEAE	3 (1.3)/3	1 (0.4)/5	4 (0.8)/8
Number of subjects with at least 1 treatment related SAE	11 (4.6)/14	5 (2.1)/7	16 (3.3)/21
Number of subjects who died	0/0	1 (0.4)/1	1 (0.2)/1
Number of subjects with at least 1 local and systemic allergic reaction	4 (1.7)/4	4 (1.7)/4	8 (1.7)/8
Number of subjects with at least 1 device related safety event	2 (0.8)/2	0/0	2 (0.4)/2

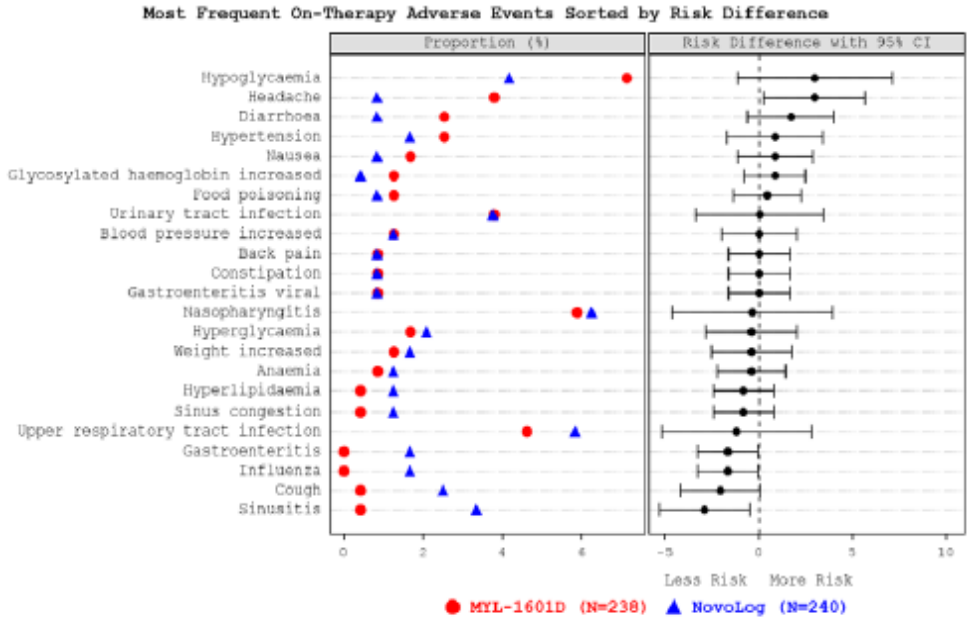
Source: Table 14.3.1.2
Abbreviations: AE = adverse events; N = number of subjects in population; n = number of subjects with event; SAE = serious adverse event; TEAE = treatment emergent adverse event.
An AE was considered a TEAE if it occurred after the first dose of study treatment medication after randomization through follow-up visit or 14 days after last dose (for subjects who did not have a follow-up visit).
An AE was considered related if causality was either definitely, probably, or possibly related. If a relationship was missing for an AE, it was assumed to have a "reasonable possibility of relatedness". If a subject had more than 1 occurrence of the same preferred term, the worst relationship was summarized.
Death included both TEAEs and non-TEAEs.
Multiple occurrences of the same adverse event in 1 individual were counted only once.
Medical Dictionary for Regulatory Activities Version 22.0 was used to code AEs.
Percentages were based on N.

Table 12-5 Summary of Twenty Most Frequent Treatment Emergent Adverse Events (Safety Analysis Set)

Preferred Term	MYL-1601D (N = 238) n (%)/#events	NovoLog (N = 240) n (%)/#events	Total (N = 478) n (%)/#events
Nasopharyngitis	14 (5.9)/15	15 (6.3)/19	29 (6.1)/34
Hypoglycemia	17 (7.1)/20	10 (4.2)/13	27 (5.6)/33
Upper respiratory tract infection	11 (4.6)/16	14 (5.8)/16	25 (5.2)/32
Urinary tract infection	9 (3.8)/10	9 (3.8)/11	18 (3.8)/21
Headache	9 (3.8)/13	2 (0.8)/2	11 (2.3)/15
Hypertension	6 (2.5)/6	4 (1.7)/4	10 (2.1)/10
Hyperglycemia	4 (1.7)/5	5 (2.1)/6	9 (1.9)/11
Sinusitis	1 (0.4)/1	8 (3.3)/10	9 (1.9)/11
Diarrhea	6 (2.5)/6	2 (0.8)/3	8 (1.7)/9
Cough	1 (0.4)/1	6 (2.5)/6	7 (1.5)/7
Weight increased	3 (1.3)/3	4 (1.7)/4	7 (1.5)/7
Blood pressure increased	3 (1.3)/5	3 (1.3)/3	6 (1.3)/8
Nausea	4 (1.7)/4	2 (0.8)/2	6 (1.3)/6
Anemia	2 (0.8)/2	3 (1.3)/3	5 (1.0)/5
Food poisoning	3 (1.3)/3	2 (0.8)/2	5 (1.0)/5
Back pain	2 (0.8)/2	2 (0.8)/3	4 (0.8)/5
Constipation	2 (0.8)/2	2 (0.8)/2	4 (0.8)/4
Gastroenteritis	0/0	4 (1.7)/4	4 (0.8)/4
Gastroenteritis viral	2 (0.8)/2	2 (0.8)/3	4 (0.8)/5
Glycosylated hemoglobin increased	3 (1.3)/3	1 (0.4)/1	4 (0.8)/4
Hyperlipidemia	1 (0.4)/1	3 (1.3)/3	4 (0.8)/4
Influenza	0/0	4 (1.7)/4	4 (0.8)/4
Sinus congestion	1 (0.4)/1	3 (1.3)/3	4 (0.8)/4

Source: Table 14.3.1.3
Abbreviations: AE = adverse event; N = number of subjects in population; n = number of subjects with event.
An AE was considered treatment emergent if it occurred after the first dose of study treatment medication after randomization through follow-up visit or 14 days after last dose (for subjects who did not have a follow-up visit).
Multiple occurrences of the same AE in 1 individual were counted only once.
The 20 most frequent events plus ties are included in the table.
Adverse events are presented in decreasing order of frequency based on the total column and then by alphabetical order (for preferred terms with same frequency).
Medical Dictionary for Regulatory Activities Version 22.0 was used to code AEs.
Percentages were based on N.

Figure 12–1 Twenty Most Frequent On-Therapy Treatment Emergent Adverse Events Sorted by Risk Difference (Safety Analysis Set)



Source: Figure 14.3.1.18
Abbreviations: CI = confidence interval; N = number of subjects in population
Twenty most frequent events plus ties are included in the figure.

The frequency of Grade 3 and 4 TEAE were 5.5% and 3.8% in the MYL-1601D group, respectively, and 4.6% and 0.8% in the NovoLog group, respectively (Table 12-6).

Table 12-6 Summary of Treatment Emergent Adverse Events (Reported by > 1 Subject in any Treatment Group) by Severity with System Organ Class and Preferred Term for Events of Grade 3 and Above (Safety Analysis Set)

System Organ Class Preferred Term	MYL-1601D (N = 238) n (%)/#events	NovoLog (N = 240) n (%)/#events	Total (N = 478) n (%)/#events
Any treatment emergent adverse event	108 (45.4)/256	103 (42.9)/231	211 (44.1)/487
Grade 1	59 (24.8)/175	52 (21.7)/137	111 (23.2)/312
Grade 2	27 (11.3)/49	36 (15.0)/71	63 (13.2)/120
Grade 3	13 (5.5)/22	11 (4.6)/15	24 (5.0)/37
Grade 4	9 (3.8)/10	2 (0.8)/6	11 (2.3)/16
Grade 5	0/0	1 (0.4)/1	1 (0.2)/1
Missing	0/0	1 (0.4)/1	1 (0.2)/1[a]
Metabolism and nutrition disorders			
Grade 3	5 (2.1)/7	9 (3.8)/11	14 (2.9)/18
Hypoglycemia	5 (2.1)/7	8 (3.3)/10	13 (2.7)/17
Grade 4	9 (3.8)/9	1 (0.4)/1	10 (2.1)/10
Hypoglycemia	9 (3.8)/9	0/0	9 (1.9)/9

Source: Table 14.3.1.7
Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects in population; n = number of subjects with event.
An AE was considered treatment emergent if it occurred after the first dose of study treatment medication after randomization through follow-up visit or 14 days after last dose (for subjects who did not have a follow-up visit).
a. AE was not graded due to patient withdrawing consent.
Multiple occurrences of the same AE in 1 individual were counted only once (under worst severity).
Adverse events were sorted alphabetically by system organ class and within each system organ class the preferred term was presented in decreasing order of frequency according to the total column.
Medical Dictionary for Regulatory Activities Version 22.0 was used to code AEs.
Severity was classified according to the CTCAE grading.
Percentages were based on N.

Table 12-7 Summary of Treatment Emergent Adverse Events by Relationship with System Organ Class and Preferred Term for Related Events (Safety Analysis Set)

System Organ Class Preferred Term	MYL-1601D (N = 238) n (%)/#events	NovoLog (N = 240) n (%)/#events	Total (N = 478) n (%)/#events
Any treatment emergent adverse event	108 (45.4)/256	103 (42.9)/231	211 (44.1)/487
Related	18 (7.6)/24	10 (4.2)/13	28 (5.9)/37
Not related	90 (37.8)/232	93 (38.8)/218	183 (38.3)/450
Related			
Blood and lymphatic system disorders	0/0	1 (0.4)/1	1 (0.2)/1
Anemia	0/0	1 (0.4)/1	1 (0.2)/1
General disorders and administration site conditions	2 (0.8)/2	1 (0.4)/1	3 (0.6)/3
Injection site bruising	0/0	1 (0.4)/1	1 (0.2)/1
Injection site pain	1 (0.4)/1	0/0	1 (0.2)/1
Injection site reaction	1 (0.4)/1	0/0	1 (0.2)/1
Investigations	3 (1.3)/4	2 (0.8)/2	5 (1.0)/6
Weight increased	2 (0.8)/2	2 (0.8)/2	4 (0.8)/4
Blood glucose increased	2 (0.8)/2	0/0	2 (0.4)/2
Metabolism and nutrition disorders	13 (5.5)/16	6 (2.5)/9	19 (4.0)/25
Hypoglycemia	12 (5.0)/15	6 (2.5)/8	18 (3.8)/23
Hyperglycemia	1 (0.4)/1	1 (0.4)/1	2 (0.4)/2
Nervous system disorders	2 (0.8)/2	0/0	2 (0.4)/2
Headache	1 (0.4)/1	0/0	1 (0.2)/1
Facial paralysis	1 (0.4)/1	0/0	1 (0.2)/1

Source: Table 14.3.1.8
Abbreviations: AE = adverse event; N = number of subjects in population; n = number of subjects with event.
An AE was considered treatment emergent if it occurred after the first dose of study treatment medication after randomization through follow-up visit or 14 days after last dose (for subjects who did not have a follow-up visit).
Multiple occurrences of the same AE in 1 individual were counted only once.

Adverse events were sorted alphabetically by system organ class and within each system organ class the preferred term was presented in decreasing order of frequency according to the total column.
An AE was considered related if causality was either definitely, probably, or possibly related. If a relationship was missing for an AE, it was assumed to have a "reasonable possibility of relatedness". If a subject had more than 1 occurrence of the same preferred term, the worst relationship was summarized.
Medical Dictionary for Regulatory Activities Version 22.0 was used to code AEs.
Percentages were based on N.

The treatment related adverse events were low in both treatment groups (7.6% vs. 4.2%), and were mostly attributed to hypoglycaemia (Table 12-7).

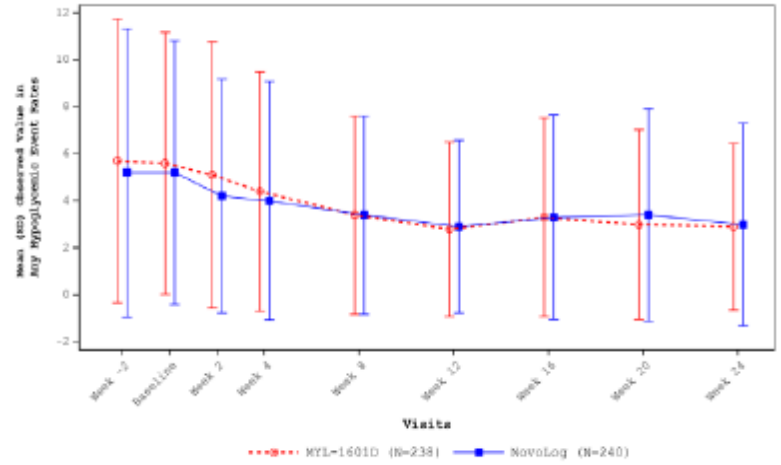
Among patients in the MYL-1601D treatment group, 17 subjects (7.1%) had an incidence of hypoglycaemia in contrast to 10 subjects (4.2%) in the NovoLog group.

The Applicant also reports that 218 (91.6%) patients in the MYL-1601D group and 222 (92.5%) patients in the NovoLog group reported a hypoglycaemic event at any visit.

The hypoglycaemic event rate and nocturnal hypoglycaemic events were similar for the two treatment groups over time (Figure 12-2 and Figure 12-4).

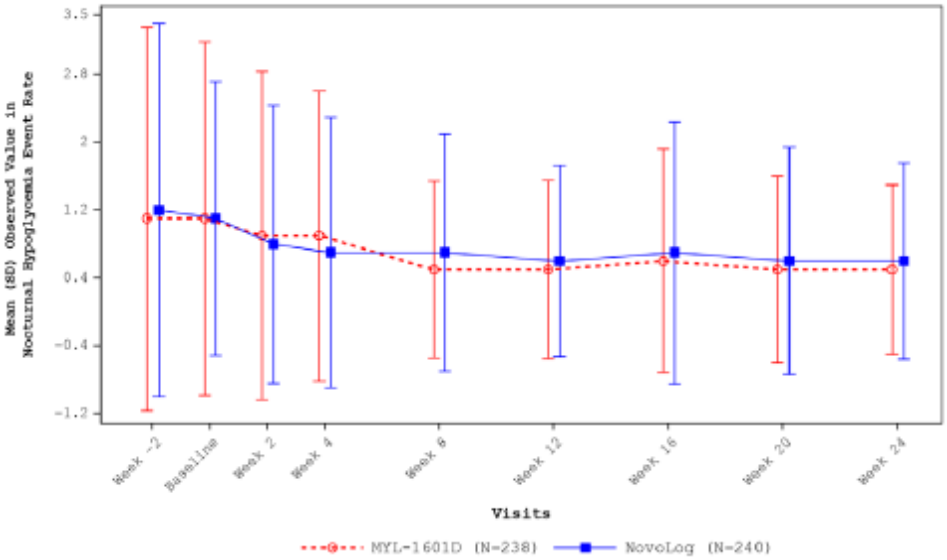
Overall, the differences in hypoglycaemic events between treatment groups might be due to the unblinded design, and the subjects in the experimental treatment arm might have had more glucose measures taken. No information regarding the number of glucose measurements were collected during the study.

Figure 12–2 Means and Standard Deviations for Hypoglycemic Event Rates (Episodes per 30 Days Adjusted) (Safety Analysis Set)



Source: Figure 14.3.1.23
Abbreviations: N = number of subjects in the population; SD = standard deviation.
Means and standard deviations are from descriptive statistics procedure.

Figure 12–4 Means and Standard Deviations for Nocturnal Hypoglycemic Event Rates (Episodes per 30 Days Adjusted) (Safety Analysis Set)



Source: Figure 14.3.1.26
Abbreviations: N = number of subjects in population; SD = standard deviation.
Means and SD are from descriptive statistics procedure.

There were no differences between treatment groups in device related adverse events, local allergic reaction, systemic allergic reactions, hypersensitivity or immune mediated adverse events. Discontinuation due to adverse events was low, and there were no relevant differences in discontinuation between treatment groups.

In one patient an abnormal ECG finding was observed at week 24. This patient had a normal ECG at baseline. The ECG showed left axis deviation, moderate voltage criteria for left ventricular hypertrophy. and left atrial hemiblock. The abnormal ECG is not considered related to the drug, rather to the disease under treatment (type 1 diabetes).

Serious adverse event/deaths/other significant events

Serious adverse events were numerically higher in patients treated with MYL-1605D compared with NovoLog. During the treatment period, 22 subjects (9.2%) in the MYL-1601D group reported 27 serious adverse events and 15 (6.3%) patients in the NovoLog group reported 23 serious adverse events (Table 5). Hypoglycaemia was the only event reported more than once per treatment group.

One patient in the NovoLog group was a victim of a homicide and died.

Table 5 Summary of Serious Treatment Emergent Adverse Events (Safety Analysis Set)

	MYL-1601D	NovoLog®	Total
System Organ Class	(N = 238)	(N = 240)	(N = 478)
Preferred Term	n (%)/#events	n (%)/#events	n (%)/#events
Any serious treatment emergent adverse event	22 (9.2)/27	15 (6.3)/23	37 (7.7)/50
Blood and lymphatic system disorders	1 (0.4)/1	0/0	1 (0.2)/1
Anemia	1 (0.4)/1	0/0	1 (0.2)/1
Gastrointestinal disorders	1 (0.4)/1	0/0	1 (0.2)/1
Colitis	1 (0.4)/1	0/0	1 (0.2)/1
General disorders and administration site conditions	1 (0.4)/1	0/0	1 (0.2)/1
Chest pain	1 (0.4)/1	0/0	1 (0.2)/1
Infections and infestations	4 (1.7)/4	2 (0.8)/4	6 (1.3)/8
Anal abscess	1 (0.4)/1	0/0	1 (0.2)/1
Cellulitis	1 (0.4)/1	0/0	1 (0.2)/1
Gastroenteritis	0/0	1 (0.4)/1	1 (0.2)/1
Incision site cellulitis	1 (0.4)/1	0/0	1 (0.2)/1
Perineal abscess	1 (0.4)/1	0/0	1 (0.2)/1
Pneumonia	0/0	1 (0.4)/1	1 (0.2)/1
Pyelonephritis	0/0	1 (0.4)/1	1 (0.2)/1
Sepsis	0/0	1 (0.4)/1	1 (0.2)/1
Injury, poisoning and procedural complications	1 (0.4)/1	1 (0.4)/1	2 (0.4)/2
Concussion	0/0	1 (0.4)/1	1 (0.2)/1
Skin laceration	1 (0.4)/1	0/0	1 (0.2)/1
Metabolism and nutrition disorders	15 (6.3)/18	11 (4.6)/13	26 (5.4)/31
Hypoglycemia	15 (6.3)/18	10 (4.2)/12	25 (5.2)/30
Diabetic ketoacidosis	0/0	1 (0.4)/1	1 (0.2)/1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0/0	1 (0.4)/1	1 (0.2)/1
Basal cell carcinoma	0/0	1 (0.4)/1	1 (0.2)/1

	MYL-1601D	NovoLog®	Total
System Organ Class	(N = 238)	(N = 240)	(N = 478)
Preferred Term	n (%)/#events	n (%)/#events	n (%)/#events
Nervous system disorders	1 (0.4)/1	1 (0.4)/1	2 (0.4)/2
Hypoglycemic seizure	1 (0.4)/1	1 (0.4)/1	2 (0.4)/2
Psychiatric disorders	0/0	1 (0.4)/1	1 (0.2)/1
Suicidal ideation	0/0	1 (0.4)/1	1 (0.2)/1
Respiratory, thoracic and mediastinal disorders	0/0	1 (0.4)/1	1 (0.2)/1
Acute respiratory failure	0/0	1 (0.4)/1	1 (0.2)/1
Social circumstances	0/0	1 (0.4)/1	1 (0.2)/1
Victim of homicide	0/0	1 (0.4)/1	1 (0.2)/1

Source: Table 14.3.1.10, MYL-1601D 3001 CSR

Abbreviations: AE = adverse event; N = number of subjects in population; n = number of subjects with event.

An AE was considered a serious treatment emergent AE if it occurred after the first dose of study treatment medication after randomization through follow-up visit or 14 days after last dose (for subjects who did not have a follow-up visit).

Multiple occurrences of the same AE in 1 individual are counted only once.

Adverse events were sorted alphabetically by system organ class and within each system organ class the preferred term was presented in decreasing order of frequency according to the total column.

Medical Dictionary for Regulatory Activities Version 22.0 was used to code AEs.

Percentages were based on N.

Laboratory findings

No major differences in laboratory parameters was identified between the two treatment groups.

Safety in special populations

Safety parameters have not been reported across age-groups. The majority of the patients were between 21 and 65 years old (N=441). In the youngest age group (<= 21 years) 25 patients were included. In the oldest age group (>= 65 years) 12 patients were included. Treatment emergent antibody response in relation to age is addressed in the efficacy section.

Immunological events

The majority of patients had positive ADA at baseline (Table 12-18). There were no differences between treatment groups in patients with newly positive ADA post-baseline or patients experiencing a 4-fold increase in antibodies.

Table 12-18 Summary of Anti-drug Antibody Response (Safety Analysis Set)

Characteristic	MYL-1601D (N = 238)	NovoLog (N = 240)	*P*-Value [a]
Subjects with ADA negative at baseline, n (%)	48 (20.2)	69 (28.8)	NA
Subjects with newly positive post-baseline ADA (treatment-induced TEAR), n (%)	24 (10.1)	35 (14.6)	0.1348
Median maximum titer	7.0	8.0	
Subjects with ADA positive at baseline, n (%)	189 (79.4)	170 (70.8)	NA
Subject with 4-fold increase in titer (treatment-boosted TEAR), n (%)	28 (11.8)	22 (9.2)	0.3534
Median titer	117.5	114.0	
Subjects with at least 1 positive ADA, n (%)	210 (88.2)	202 (84.2)	0.1973
Subjects with TEAR, n (%)	52 (21.8)	57 (23.8)	0.6203
Subjects without TEAR, n (%)	163 (68.5)	157 (65.4)	0.4755
Median time to TEAR (days)	196.5	195.0	NA
Subjects with Unconfirmed TEAR status[b], n (%)	23 (9.7)	26 (10.8)	0.6734
Subjects who met TEAR, HbA1c, and dose criteria at any visit, n (%)	14 (5.9)	12 (5.0)	0.6706

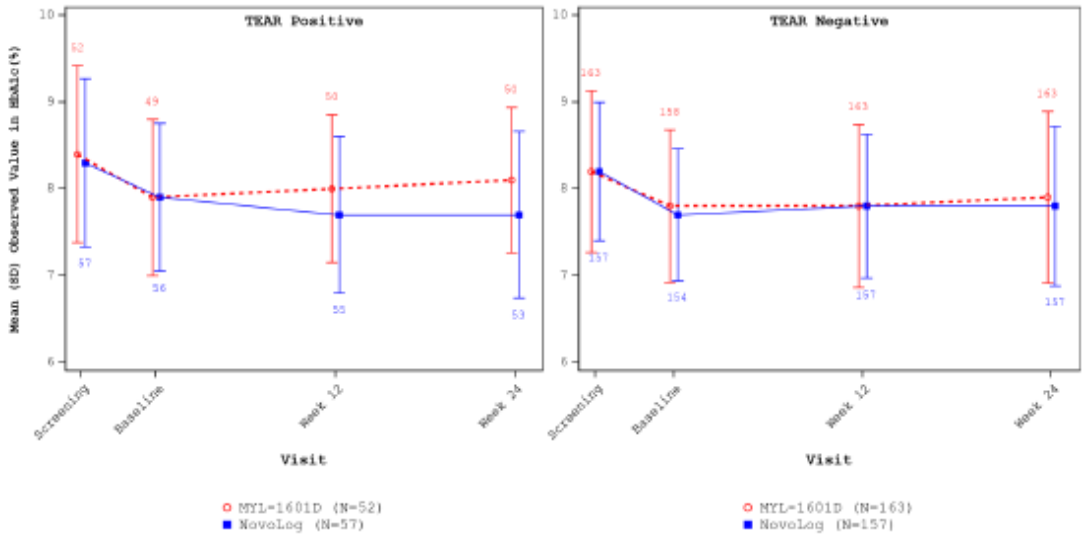
Source: Table 14.3.8.4
Abbreviations: ADA = anti-drug antibody; HbA1c = glycosylated hemoglobin; N = number of subjects in population; n = number of subjects with data; NA = not applicable; TEAR = treatment emergent antibody response.
a *P*-value was based on Chi-Square test if 80% of the cells had an expected frequency ≥ 5, Fisher's exact test was used otherwise.
b Subjects with at least 1 missing scheduled visit data and TEAR positive status could not be determined.
Percentages were based on N.

In patients with TEAR post-baseline, the incidence of neutralising antibodies was low and comparable between groups. As such, the 4 patients (7.7%) in the MYL-1601D had neutralising antibodies and 4 patients (7.0%) in the Novolog group had neutralising antibodies. In patients who were TEAR negative, 24 patients (14.7%) in the MYL-1601D group and 20 patients (12.7%) in the NovoLog group had neutralising antibodies.

HbA_{1c}, insulin dose, hypoglycaemic events and injection site reactions/hypersensitive were analysed by TEAR status. There was a tendency towards a small increase in HbA_{1c} for MYL-1601D and a small decrease in HbA_{1c} for Novolog in TEAR positive patients, whereas there was no difference between treatment group in TEAR negative patients. The difference in HbA_{1c} at week 24 between MYL-1601D and NovoLog in TEAR positive patients was 0.26%-point (95% CI: -0.03, 0.56). The differences at 26 weeks could be due to a minor decrease in daily insulin dose during the first 4 weeks in the MYL1601D group, which is not considered caused by TEAR. The difference is not statistically significant and is not considered clinically relevant (Figure 12-6).

There were no differences in daily insulin dose at week 26 (Figure 12-7), hypoglycaemic events (Figure 12-8), injection site reactions or hypersensitivity between treatment groups in TEAR positive patients.

Figure 12–6 HbA1c (%) Profiles Mean (Standard Deviation) for Treatment Emergent Antibody Response Status by Treatment Group (Safety Analysis Set)



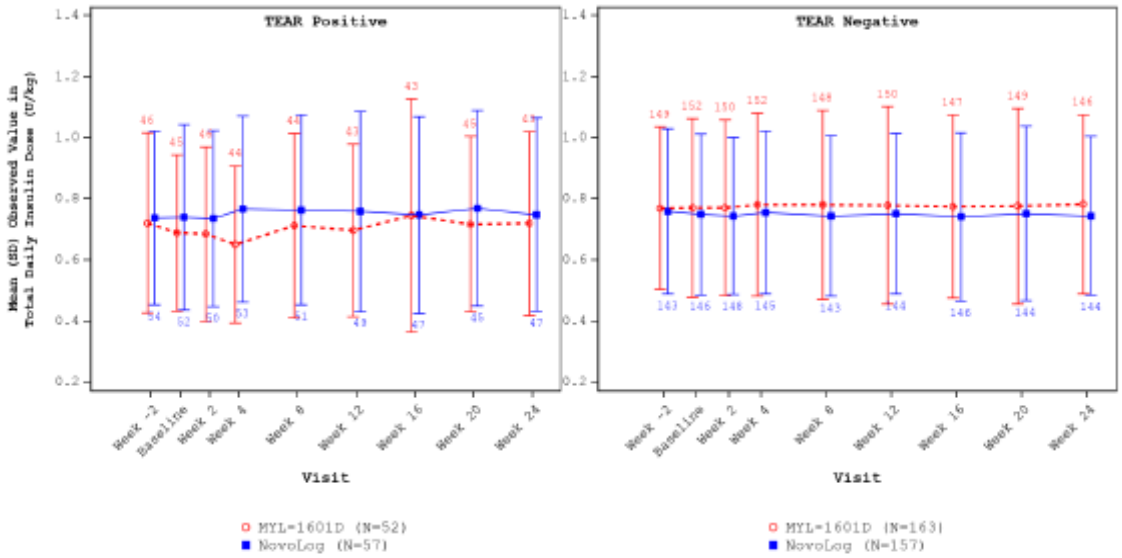
Source: Figure 14.3.8.8.2

Abbreviations: ADA = anti drug antibody; HbA1c = glycosylated hemoglobin; N = number of subjects in population; SD = standard deviation; TEAR = Treatment Emergent Antibody Response.

TEAR positive was defined as either 1 of the following:

1) Subjects who were ADA negative at baseline and become positive at any timepoint post-baseline.
2) Subjects who were ADA positive at baseline and demonstrate 4-fold increase in titer values at any timepoint post-baseline visit.

Figure 12–7 Total Daily Insulin Dose (U/kg) Profiles Mean (Standard Deviation) by Treatment Emergent Antibody Response Status (Safety Analysis Set)



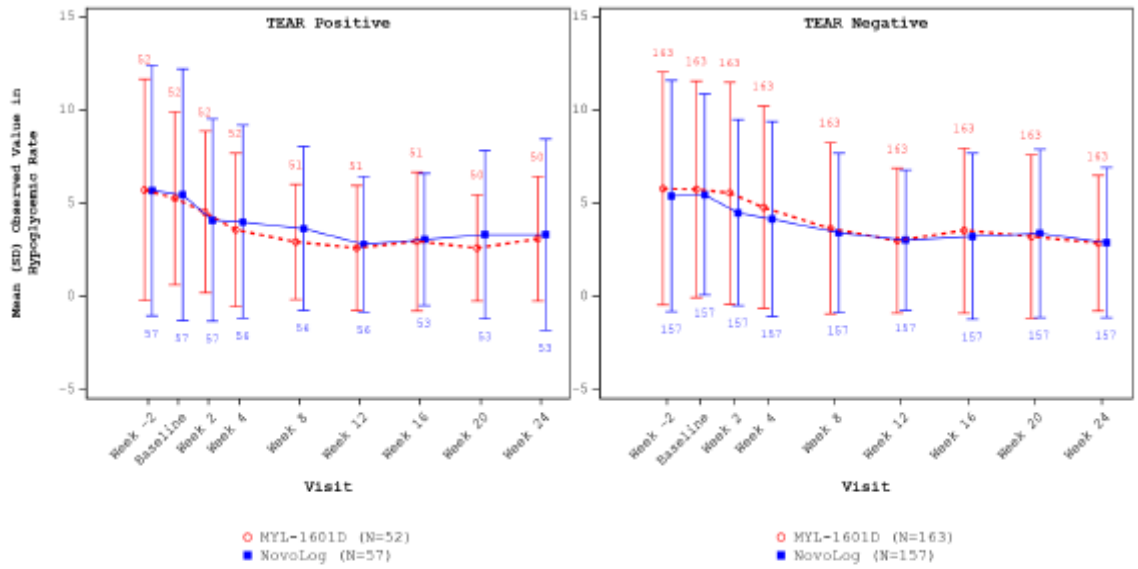
Source: Figure 14.3.8.9.2

Abbreviations: ADA = anti drug antibody; N = number of subjects in population; SD = standard deviation; TEAR = Treatment Emergent Antibody Response.

TEAR positive was defined as either 1 of the following:

1) Subjects who were ADA negative at baseline and become positive at any timepoint post-baseline.
2) Subjects who were ADA positive at baseline and demonstrate 4-fold increase in titer values at any timepoint post baseline visit.

Figure 12–8 Hypoglycemic Rate (Episodes per 30 Days Adjusted) Profiles Mean (Standard Deviation) by Treatment Emergent Antibody Response Status (Safety Analysis Set)



Source: Figure 14.3.8.10.2
Abbreviations: ADA = anti drug antibody; N = number of subjects in population; SD = standard deviation; TEAR = Treatment Emergent Antibody Response.
TEAR positive was defined as either 1 of the following:

1. Subjects who were ADA negative at baseline and become positive at any timepoint post baseline.
2. Subjects who were ADA positive at baseline and demonstrate 4-fold increase in titer values at any timepoint post baseline visit.

Safety related to drug-drug interactions and other interactions

N/A

Post marketing experience

N/A

Study MYL-1601D-1001

Adverse events

*In study MYL-1601D-1001, 11 subjects (15.7%) experienced at least one TEAE after administration of MYL-*1601D, 13 subjects (19.4%) after administration of NovoRapid® and 11 subjects (15.9%) after administration of NovoLog®. Overall, 18, 13 and 12 TEAEs were reported for MYL-1601D, NovoRapid® and NovoLog®, respectively. Most of the 43 TEAEs were of mild (24 events) or moderate (18 events) intensity. One TEAE was severe (influenza, which was unrelated to IMP). All TEAEs had the outcome recovered/resolved.

The number of drug-related TEAEs was comparable between treatments (4 events for MYL-1601D, 5 events for NovoRapid® and 3 events for NovoLog®). These events included moderate nausea (1 event), injection site erythema (4 mild events), hypoglycemia (2 mild events) and headache (5 events).

Table 30 Summary of Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term (Safety Analysis Set)

		Number (%) of Subject / Number of Events		
System Organ Class	Preferred Term	MYL-1601D (N=70)	NovoRapid (N=67)	NovoLog (N=69)
Overall		11 (15.7%)/18	13 (19.4%)/13	11 (15.9%)/12
Gastrointestinal disorders	Constipation	1 (1.4%)/ 1	0	0
	Eructation	1 (1.4%)/ 1	0	0
	Nausea	0	1 (1.5%)/ 1	0
General disorders and administration site conditions	Decreased appetite	1 (1.4%)/ 1	0	0
	Injection site erythema	2 (2.9%)/ 2	1 (1.5%)/ 1	1 (1.4%)/ 1
Infections and infestations	Influenza	0	0	1 (1.4%)/ 1
	Nasopharyngitis	3 (4.3%)/ 3	2 (3.0%)/ 2	0
Injury, poisoning and procedural complications	Contusion	1 (1.4%)/ 1	0	0
	Infusion site extravasation	1 (1.4%)/ 1	0	1 (1.4%)/ 2
	Infusion site pain	0	0	1 (1.4%)/ 1
Metabolism and nutrition disorders	Hypoglycaemia	1 (1.4%)/ 1	0	1 (1.4%)/ 1
Musculoskeletal and connective tissue disorders	Neck pain	0	0	1 (1.4%)/ 1
Nervous system disorders	Headache	4 (5.7%)/ 4	8 (11.9%)/ 8	5 (7.2%)/ 5
	Tension headache	0	1 (1.5%)/ 1	0
Skin and subcutaneous tissue disorders	Rash	1 (1.4%)/ 1	0	0
	Skin abrasion	1 (1.4%)/ 1	0	0
Vascular disorders	Syncope	1 (1.4%)/ 1	0	0

Unique events for a subject are counted once within each preferred term
Calculation of percent is based on number of subjects exposed to the respective IMP.
Coding according to MedDRA Version 20.1
Cross-reference: EOT Table 14.3.1.2

Serious adverse events and deaths

No SAEs or deaths occurred in study MYL-1601D-1001.

Laboratory findings

Among the individual hematology, biochemistry and urinalysis parameters, no value was assessed as clinically significant requiring reporting as AE.

Immunological events

A total of 5 subjects (of 72 subjects) had confirmed positive ADA sample results at any time point during the MYL-1601-1001 study. One subject (subject 030) had a treatment-induced antibody response, while 4 subjects were already positive at visit 2a, prior to dosing. The relative ADA levels in all positive samples was low, ranging between 4.78 and 7.59 %/bound/total.

Subject 030 was ADA negative at visit 2a (baseline), but positive on visit 2b following administration of MYL-1601D, and returned to negative at visit 3. At visit 2b, the percent inhibition after incubation with each drug product was 35.20%, 36.45%, and 36.14% for MYL- 1601D, NovoLog® and NovoRapid®, respectively. Comparability of the percent inhibition values indicates that the ADA present in this sample was cross-reactive with each drug product and arose from a common epitope. No cross-reactivity to human insulin was observed for this sample. The subject had PK and PD parameters similar to the other subjects and only one AE (influenza), reported several days after visit 3. In summary, immunogenicity assessments did not show an increased ADA reactivity for MYL-1601D compared to NovoLog® or NovoRapid®, since the majority of subjects lacked any detectable immunogenicity response against insulin aspart or had relevant antibody levels pre-dose at visit 2a with no treatment boosting. Only one subject had a treatment-induced transient ADA reaction to MYL-1601D, with a similar percent inhibition for MYL-1601D, NovoLog® and NovoRapid®. For further information related to the assessment of the novel proinsulin impurity and its impact on safety please refer to section "2.6.1 Discussion on clinical safety" below.

Discontinuation due to AES

None of the AEs resulted in a discontinuation of study drug.

2.6.1. Discussion on clinical safety

The clinical study program for MYL-1601D includes two clinical studies, namely study MYL-1601D-1001 (including single-dosing of the three IMPs in 71 healthy subjects) and study MYL-1601D-3001. These study designs imply that safety evaluation should mainly be based on the MYL-1601D-3001 study. The safety database is considered acceptable.
It is acknowledged that safety studies for similar biological medicinal products containing insulin analogues should be performed with specific focus on immunogenicity (EMEA/CHMP/BMWP/32775/2005_Rev. 1), and the clinical study program for MYL-1601D is considered reasonable. Overall, the provided preliminary safety evaluation based on data from study MYL-1601D-1001 is considered to be of limited value due the single-dose application of MYL-1601D in this study.
The proposed primary objective of study MYL-1601D-3001 is to demonstrate that immunogenicity as assessed by treatment emergent antibody response (TEAR) rate with MYL-1601D is equivalent to that of NovoLog® during 24-week treatment. The "Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues" [EMEA/CHMP/BMWP/32775/2005_Rev. 1] recommends a duration of 6 months for immunogenicity studies of biosimilar insulins as anti-drug antibodies are expected to develop early-on.
However, Scientific Advice received stated that the current issue is not the immunogenicity of aspart insulin but the immunogenicity of the novel proinsulin impurity. The Applicant has discussed that the proinsulin impurity is present only at very low levels in the MYL-1601D drug product, and the risk of antibody generation against this impurity is considered low.

Study 3001 was a randomised, multicentre, open-label, parallel-group clinical study comparing the safety, immunogenicity, and efficacy of MYL-1601D with NovoLog in Type 1 Diabetes Mellitus (T1DM) patients.
The safety population from study 3001 consisted of 478 patients of which 238 had at least one dose of MYL-1601-D. The baseline characteristics were similar between treatment groups. Overall, the characteristics were well balanced between treatment groups, and the population included in study 3001 is adequately reflecting an adult type 1 diabetes population.

Adverse events
Overall, the number of subjects experiencing an adverse event, and the number of events were higher in the MYL-1601D group than in the NovoLog group. The difference is considered small (108 patients out of 238 vs 105 patients out of 240). The Applicant discusses that the higher frequency of adverse events can be attributed to the open label study design, which is agreed. Hypoglycaemia was the most frequent adverse event and was also the most frequent adverse event related to the treatment.
Among patients in the MYL-1601D treatment group, 17 subjects (7.1%) had an incidence of hypoglycaemia in contrast to 10 subjects (4.2%) in the NovoLog group.
The Applicant also reports that 218 (91.6%) patients in the MYL-1601D group and 222 (92.5%) patients in the NovoLog group reported a hypoglycaemic event at any visit.
The hypoglycaemic event rate and nocturnal hypoglycaemic events were similar for the two treatment groups over time.
Overall, the differences in hypoglycaemic events between treatment groups might be due to the unblinded design, and the subjects in the experimental treatment arm might have had more glucose measurements taken. No information regarding the number of glucose measurements were collected during the study.
There were no differences between treatment groups in device related adverse events, local allergic reaction, systemic allergic reactions, hypersensitivity or immune mediated adverse events. Discontinuation due to adverse events was low, and there were no relevant differences in discontinuation between treatment groups.
In one patient an abnormal ECG finding was observed at week 24, and the abnormal ECG finding is not considered related to the drug, rather to the disease under treatment (type 1 diabetes).

The frequency of Grade 3 and 4 TEAE were 5.5% and 3.8% in the MYL-1601D group, respectively, and 4.6% and 0.8% in the NovoLog group, respectively. The higher frequency in the MYL-1601D group is considered due to the open label design of the study.
Safety parameters have not been reported across age-groups. Treatment emergent antibody response in relation to age is addressed in the efficacy section. This is acceptable.

Serious adverse events
Serious adverse events were numerically higher in patients treated with MYL-1605D compared with NovoLog. During the treatment period, 22 subjects (9.2%) in the MYL-1601D group reported 27 serious adverse events and 15 (6.3%) patients in the NovoLog group reported 23 serious adverse events. Hypoglycaemia was the only event reported more than once per treatment group. The Applicant states that 10 of the patients in the MYL-1601D treatment group and 5 patients in the NovoLog group had contributing factors to hypoglycaemia, e.g. excessive physical activity, irregular diet, alcohol consumption, or not taking medication/incorrect dose. This is considered acceptable.
One patient in the NovoLog group was a victim of a homicide and died.

Immunological events

The majority of patients had positive ADA at baseline. There were no differences between treatment groups in patients with newly positive ADA post-baseline or patients experiencing a 4-fold increase in antibodies. In conclusion, there were no differences in neutralising antibodies between treatment groups and the incidence of neutralising antibodies was low, and no differences in the incidence of neutralising antibodies with respect to TEAR status were observed.
HbA_{1c}, insulin dose, hypoglycaemic events and injection site reactions/hypersensitive were analysed by TEAR status. There was a tendency towards a small increase in HbA_{1c} for MYL-1601D and a small decrease in HbA_{1c} for Novolog in TEAR positive patients, whereas there was no difference between treatment group in TEAR negative patients. The difference between treatment arms among TEAR positive patients is not considered clinically relevant and is likely to be caused by an initial lower daily insulin dose in the MYL-1601D group. There were no overall differences in daily insulin dose, hypoglycaemic events, injection site reactions or hypersensitivity between treatment groups in TEAR positive patients.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

2.6.2. Conclusions on the clinical safety

The safety of MYL-1601D has been well described.

Overall, TEAR did not differ substantially between treatment groups and there was no impact of TEAR on effect parameters.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

Pharmacovigilance plan

No additional pharmacovigilance activities are recommended. Routine pharmacovigilance is considered sufficient to identify and further characterise the risks of the medicinal product.

Risk minimisation measures

As there are no important identified/potential risks or missing information included as part of the safety specification of the RMP, routine risk minimisation measures are considered sufficient for this medicinal product.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.2 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.9. Product information

2.9.1. User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the applicant and has been found acceptable for the following reasons:

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to NovoRapid 100units/ml. The bridging report submitted by the applicant has been found acceptable.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Kixelle (insulin aspart) is included in the additional monitoring list as it is a biological substance authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

This application is a centralised procedure made according to Article 10(4), Directive 2001/83/EC, biosimilar application. MYL-1601D, insulin aspart, solution for injection, has been developed as a biosimilar of

NovoRapid/Novolog (insulin aspart 100 U/ml) which is marketed by Novo Nordisk A/S and authorized in the EU since 1999. The Applicant is seeking approval for the same indication as NovoRapid:

"Kixelle is indicated for treatment of diabetes mellitus in adults, adolescents and children aged 1 year and above."

Demonstration of biosimilarity of MYL-1601D to the reference product NovoRapid/NovoLog was based on in vivo and in vitro studies, non-clinical studies, a human PK/PD study and a phase 3 immunogenicity study, as described below.

Quality

Insulin aspart is a relatively small and less complex protein molecule enabling thorough and reliable comparison of quality attributes covering structure and impurity pattern. The finished product formulations of MYL-1601D and the reference product NovoRapid® are highly similar.

The MYL-1601D solution for s.c. injection is presented in cartridges and pre-filled pens, which are also the common presentations of the reference product.

A comprehensive analytical comparability study was performed to evaluate the similarity between MYL-1601D and the reference product NovoRapid®.

The Applicant's strategy to establish similarity of MYL-1601D with the reference product is endorsed. The attributes tested in the biosimilarity study focus in particular on structure, impurity pattern and biological activity and they cover all attributes relevant for the analytical comparison.

A considerable number of batches have been analysed and the number of finished product batches chosen for each test is appropriate to accommodate the expected variability of the analytical methods.

The MYL-1601D DP batches included in the analytical studies are considered sufficiently representative for the commercial product. Their age at the time of testing is generally lower compared to the age of the reference batches of the EU sourced and the US sourced products. The comparative stability and forced degradation studies indicate comparable degradation rates of MYL-1601D and the reference product.

Quantitative results for the parameters tested have been presented in clear graphs where the results from all three products (MYL-1601D, NovoRapid and NovoLog) are presented. This graphical presentation enables a satisfactory comparison of results.

Biosimilarity between Kixelle and NovoRapid/NovoLog has been demonstrated.

Non-clinical

In the non-clinical part of the assessment, *in vitro* pharmacology studies are vital to show biosimilarity, whereas *in vivo* studies should only be performed when needed.

In the current MAA, a sufficient number of *in vitro* pharmacology studies have been performed in order to assess receptor binding, receptor phosphorylation and metabolic activity for MYL-1601D insulin aspart and NovoRapid (and NovoLog) in accordance with guideline (EMEA/CHMP/BMWP/ 32775/2005 Rev. 1, 2015). The methods used in the *in vitro* pharmacology studies are acceptable and sensitive enough to detect difference between the test and the reference compounds. The number of tested batches and the number of runs/replicates are sufficient. Additionally, the batch used in the clinical study MYL-1601D-1001 was tested in all *in vitro* studies. In addition to the conducted *in vitro* pharmacology studies, the current MAA also included

two in vivo toxicity studies. However, *in vivo* toxicity studies are not a requirement according to EMA guidelines due to the low sensitivity of the studies for conclusion on biosimilarity and hence the animal studies are unnecessary from a 3R perspective (EMEA/CHMP/BMWP/ 32775/2005_Rev.1).

Clinical

The clinical development consisted a single dose PK/PD euglycaemic clamp study in 72 healthy volunteers comparing MYL-1601D with NovoRapid (EU approved) and NovoLog (US licenced) (Study MYL-1601D-1001).

The PK/PD study was conducted in accordance with EMA Guideline on the non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues (EMEA/CHMP/BMWP/32775/2005_1). Furthermore, a phase 3 immunogenicity and efficacy study (24-week, open label, randomised, parallel-group) in type 1 diabetes patients comparing MYL-1601D with NovoLog was submitted with the day 121 response (Study MYL-1601D-3001).

3.2. Results supporting biosimilarity

Quality

Structural biosimilarity between the MYL-1601D test product and NovoRapid reference product and the comparator NovoLog was studied by applying numerous state-of-the-art methods. They all demonstrate similarity in terms of primary (i.e., amino acid sequence), secondary, tertiary structure and molecular mass. Likewise, protein content and pI of the MYL-1601D test product was shown to fall into the quality range of NovoRapid reference product and to be comparable between NovoRapid and the comparator NovoLog.

Purity and impurity of MYL-1601D DP, NovoRapid and NovoLog has been assessed. Determination of HMWPs concluded that the level of HMWPs is similar in MYL-1601D compared to the levels found in the reference product. This is considered acceptable.

A novel proinsulin impurity was detected in MYL-1601D. This impurity cannot be found in the EU reference product. Insulin The novel impurity is being monitored and controlled during downstream process of the MYL-1601D DS manufacturing process.

Biological activity in terms of *in vitro* metabolic and *in vitro* mitogenic activity was investigated. The results of the similarity studies concerning the biological activity of the product, demonstrated similar biological activity. The biological activity of MYL-1601D is therefore considered to be similar to that of NovoRapid.

The preservatives m-cresol and phenol are present in the same concentrations in MYL-1601D compared to the reference product. The pH of MYL-1601D batches is very close to the physiological pH.

Non-clinical

The Applicant has performed comparative receptor binding studies on the two human insulin receptors (IR-A and IR-B), including on-off kinetics, with the product under review MYL-1601D and the reference product NovoRapid (and NovoLog). Additionally, receptor binding assays were also performed for the IGF-1 receptor. Biological activity was measured by autophosphorylation of IR-A and IR-B, by inhibition of lipolysis, stimulation of glucose uptake, adipogenesis and mitogenesis of Saos2 cells.

The choice of studies is in accordance with the guideline (EMEA/CHMP/BMWP/ 32775/2005 Rev. 1, 2015). The mean, standard deviation and comparability range of the tested batches of the EU reference product

NovoRapid, NovoLog and MYL-1601D was presented in the table below. Minimum and maximum potency values of MYL-161D were within the lower and upper limit for the EU reference product NovoRapid (and NovoLog). The only exception was the upper limit of the inhibition of stimulated lipolysis which diverged slightly compared to the upper limit of NovoRapid. Overall, this was considered sufficient to support biosimilarity from a non-clinical point of view.

Table 1: Mean, Standard Deviation, and Comparability Range of Tested Batches of NovoRapid®, NovoLog®, and MYL-1601D for *in vitro* Assays

Assay	Test Article	Mean	SD	Lower Limit*	Upper Limit**
IR-A Phosphorylation (relative potency)	NovoRapid®	1.08	0.05	0.93	1.23
	NovoLog®	1.12	0.08	0.89	1.35
	MYL-1601D	1.10	0.04	1.01	1.13
IR-B Phosphorylation (relative potency)	NovoRapid®	1.08	0.10	0.76	1.39
	NovoLog®	1.09	0.08	0.86	1.32
	MYL-1601D	1.12	0.07	0.99	1.21
Glucose Uptake (relative potency)	NovoRapid®	1.11	0.17	0.61	1.60
	NovoLog®	1.07	0.12	0.70	1.43
	MYL-1601D	1.00	0.09	0.82	1.13
Adipogenesis (relative potency)	NovoRapid®	96.00	8.87	69.00	122.00
	NovoLog®	101.00	3.33	91.00	111.00
	MYL-1601D	98.00	5.80	87.00	106.00
Inhibition of Stimulated Lipolysis (relative potency)	NovoRapid®	0.96	0.11	0.64	1.27
	NovoLog®	1.05	0.08	0.81	1.28
	MYL-1601D	1.11	0.14	0.93	1.30
Mitogenic Assay (relative potency)	NovoRapid®	0.96	0.08	0.72	1.21
	NovoLog®	0.98	0.10	0.68	1.28
	MYL-1601D	1.03	0.05	0.93	1.12
IGF-1R Binding Kinetics K_D, µM	NovoRapid®	2.15	0.28	1.30	2.99
	NovoLog®	2.07	0.25	1.32	2.83
	MYL-1601D	2.29	0.22	2.04	2.68
IR-B Binding Kinetics K_D, nM***	NovoRapid®	5.65	0.49	4.14	7.10
	NovoLog®	5.75	0.64	3.84	7.66
	MYL-1601D	5.72	0.49	4.89	6.85
IR-A Binding Kinetics K_D, nM***	NovoRapid®	6.64	0.41	5.41	7.86
	NovoLog®	6.73	0.57	5.03	8.43
	MYL-1601D	6.55	0.20	6.19	6.82

Abbreviation: SD: standard deviation
*For NovoRapid® and NovoLog® samples, lower limit represents the mean minus 3 times the standard deviation while for MYL-1601D the lower limit represents the minimum measured potency value
** For NovoRapid® and NovoLog® samples, upper limit represents the mean plus 3 times the standard deviation while for MYL-1601D the upper limit represents the maximum measured potency value
***In addition to K_D, k_a and k_d were also evaluated with comparability ranges reported in Section 3.2.R.1

Clinical

For the primary PK endpoints, $AUC_{(0\text{-}12h)}$ and C_{max}, the 90% confidence intervals were within the predefined limits (80% and 125%) for the comparison with NovoLog as well as NovoRapid. Thus, similar pharmacokinetics between MYL-1601D, NovoLog (US-licensed) and NovoRapid (EU-approved) were documented.

For one of the secondary PK endpoints, $AUC_{(6\text{-}12h)}$ the ratio between MYL-1601D and NovoLog was 75.60 (61.38; 92.16), and the ratio between MYL-1601D and NovoRapid was 81.37 (62.70; 105.75) and therefore outside the predefined limits of biosimilarity. The Applicant argues that high variability was present at the end of the clamp study due to a limited number of measurements. This argument is agreed. The study met

its primary endpoints, other secondary endpoints are supportive and the results of $AUC_{(6\text{-}12h)}$ do not alone question biosimilarity. AUC measures for all other timepoints were within the 80%-125 % limits.

The mean $T_{½}$ for MYL-1601D, NovoRapid and NovoLog were 0.88 h, 0.89 h and 0.93 h, respectively, and are considered similar.

The 90% and 95% confidence intervals of the primary PD endpoints ($AUC_{GIR,0h\text{-}last}$ and GIR_{max}) lie within the prespecified range, demonstrating similar glucose lowering effect of MYL-1601D and NovoLog, and NovoRapid, respectively. No differences in glucose infusion rate between MYL-1601D and NovoRapid at the end of the clamp were observed.

The secondary end-points: time to maximum glucose infusion rate, time until 50% of $GIR_{max(early)}$, time until 50% of $GIR_{max(late)}$, onset of action and duration of action did not differ substantially between MYL-1601D, NovoRapid and NovoLog.

The Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues (EMEA/CHMP/BMWP/32775/2005_Rev. 1) declares no anticipated need for specific efficacy studies, since endpoints (usually HbA_{1c}) used in such studies are not considered sensitive enough to detect potentially clinically relevant differences between two insulins. Due to a novel proinsulin impurity a phase III immunogenicity study was requested. The 24-week immunogenicity study showed no differences in treatment emergent antibody response between the MYL-1601D group and the NovoLog group and no differences in HbA1c, fasting plasma glucose or insulin requirements during the follow-up between the two treatment groups.

Insulin aspart (NovoRapid) was first approved by European Medicine Agency (EMA) in 1999. The safety and tolerability profile for insulin aspart is considered well-established based on clinical trials and the use in diabetes patients since marketing authorization. The RMP version 3.1 for insulin aspart (NovoRapid and NovoMix) dated January 2018 includes no important identified or potential risks for NovoRapid. It is described that the important risks are well described in the proposed product information and appropriately managed by routine pharmacovigilance activities.

3.3. Uncertainties and limitations about biosimilarity

Quality

The analytical studies in 3.2R present evidence to conclude similarity of MYL-1601D and NovoRapid®.

Non-clinical

In the current MAA, non-clinical biosimilarity has been confirmed between MYL-1601D and the EU reference NovoRapid (and NovoLog) based on the *in vitro* pharmacology studies for receptor binding and metabolic activity in accordance with ICH guidelines.

It should, however, be noted that the relative potencies of the compounds were calculated toward an internal working reference standard (IWRS) instead of comparing data head-to-head as stated in the guideline (EMEA/CHMP/BMWP/ 32775/2005_Rev. 1). However, EC50/IC50 values of the functional *in vitro* pharmacology studies were provided, and showed visual overlap of EC50 values of MYL-1601D, NovoRapid, NovoLog and the IWRS in the different assays. Additionally, individual concentration-effect curves of each biological activity assay, all showed the classic sigmoid shape and the test compound curve overlapped with

that of the IRWS. Furthermore, the IWRS have been sufficiently characterized in order to assess comparability between MYL-1601D, NovoRapid® and NovoLog®. Therefore, the totality of the data provided was considered sufficient to support non-clinical biosimilarity and the issue of the lacking head-to-head comparison will not be further pursued.

Clinical

No uncertainties regarding the comparability margins for PK and PD in the euglycaemic clamp study exist.

The safety study was performed in order to clarify the immunogenicity of MYL-1601D. However, other safety parameters were also measured. There was a difference in hypoglycaemic events between the treatment groups, with a higher frequency in the MYL-1601D group. This is considered due to the unblinded design.

3.4. Discussion on biosimilarity

Non-clinical

In conclusion, non-clinical biosimilarity has been confirmed between MYL-1601D and the EU reference NovoRapid based on the conducted *in vitro* studies in accordance with ICH guidelines (EMEA/CHMP/ BMWP/32775/2005 Rev. 1, 2015).

Clinical

A single dose euglycaemic clamp study investigated the equivalence in pharmacokinetics and pharmacodynamics between MYL-1601D and NovoRapid (EU-approved) and NovoLog (US-Licensed). The study was conducted in healthy subjects and was conducted in accordance with guidelines. The study was considered well conducted.

The 90% confidence interval of the primary PK end-points ($AUC_{(0\text{-}12h)}$ and C_{max}) were within the predefined limits (80%-125%) for the comparison with NovoLog (US-licensed) as well as NovoRapid (EU-approved). Thus, similar pharmacokinetics between MYL-1601D, NovoLog and NovoRapid were documented. At the end of the clamp, high variability was observed, and AUC was lower for MYL-1601D than Novorapid and NovoLog. This finding does not alone preclude biosimilarity.

Two primary pharmacodynamic endpoints (area under the glucose infusion rate curve from 0 hours until the end of clamp and the maximum glucose infusion rate), and other relevant secondary endpoints were investigated. The 95% confidence interval of the primary PD endpoints lie within the predefined range (80%-125%) for the comparison with NovoRapid, which is in accordance with the EMA guideline. For the comparison with NovoLog, the 90% confidence interval was used due to the FDA requirements and was within the predefined range (80%-125%).

Overall, the PK/PD study was well conducted and similar pharmacokinetic and pharmacodynamic profiles have been demonstrated of MYL-1601D, NovoLog and NovoRapid. The totality of clinical PK/PD data support biosimilarity between MYL-1601D and NovoLog (US-licensed) and NovoRapid (EU-approved).

A 24-week, randomised, open label immunogenicity study was conducted comparing MYL-1601D with NovoLog (Study MYL-1601D-3001). No differences in treatment emergent antibody response were demonstrated between the treatment arms. However, a slight tendency towards increase in HbA1c in TEAR positive patients in MYL-1601D arm vs Novolog arm was observed, which is considered to be a consequence

of a slightly lower insulin dose during the first 4 weeks in the MYL-1601D arm and not a consequence of TEAR positivity.

In conclusion, clinical biosimilarity has been confirmed between MYL-1601D and NovoLog® in accordance with regulatory guidance.

3.5. Extrapolation of safety and efficacy

Not applicable.

3.6. Additional considerations

Not applicable.

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Kixelle is considered biosimilar to NovoRapid. Therefore, a benefit/risk balance comparable to the reference product can be concluded.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that Kixelle is not similar to Amglidia within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Kixelle is favourable in the following indication:

"Kixelle is indicated for treatment of diabetes mellitus in adults, adolescents and children aged 1 year and above."

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.