EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 February 2025
EMA/CHMP/340099/2025
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

Insulin Human Rechon

International non-proprietary name: insulin human

Procedure No. EMEA/H/C/006011/0000

Note

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

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List of abbreviations

Quality

aa (AA)	Amino acid
API	Active pharmaceutical ingredient
C	Celsius
CD	Circular dichroism
CFU/mL	Colony forming units/milliliter
CM	Carboxymethyl cellulose cation exchange chromatography
CPP	Critical process parameter
CQA	Critical quality attribute
CV	column volume
Da	Dalton
DNA	deoxyribonucleic acid
DO/DO_2	Dissolved oxygen
DP	Drug product
DS	Drug substance
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EPCB	End of production cell bank
EU	European Union
G	Gram
g/L	gram/liter
GMP	Good manufacturing practice
H	Hour
HMWP	High molecular weight protein
HCP	Host cell-derived protein
HPLC	High performance liquid chromatography
IB	Inclusion bodies
IEF	Iso-electric focusing
INN	International nonproprietary name
IPC	In-process control
IR	Insulin receptor
KPA	Key performance attribute
KPP	Key process parameter
L	Liter(s)
LC	Liquid chromatography
LC-ESI-MS	Liquid Chromatography-Electrospray ionization-Mass Spectroscopy
LC-MS	Liquid Chromatography Mass Spectrometry
LIVCA	limit of in-vitro cell age
MAA	Marketing authorization application
MCB	Master cell bank
mM	Milimolar
µm	Micrometer
Min	Minute(s)
MS	Mass spectroscopy
nM	Nanometer
NOR	Normal operating range
OD	Optical density
PAR	Proven acceptable range
Ph. Eur.	European pharmacopoeia
pI	Isoelctric point
PP	Process parameter
QTPP	Quality target product profile
rDNA	Recombinant deoxyribonucleic acid
RH	Room humidity
RP-HPLC	Reverse phase high performance liquid chromatography
RRT	Relative retention time
S	Second(s)

SCP	single chain precursor
US	Unites States
UV	Ultraviolet
WCB	Working cell bank
WFI	Water for injection

Non-clinical

ADME:	Absorption, Distribution, Metabolism and Excretion
AS160:	Akt Substrate
AUC:	Area under the curve
BSA:	Bovine Serum Albumin
Cmax:	Peak plasma concentration Maximum concentration
Eu Pharm:	European Pharmacopoeia
Frel:	Relative bioavailability
GLP:	Good Laboratory Practices
IGF1R:	Insulin-like growth factor 1 receptor
IR:	Insulin receptor
IRS-1:	Insulin Receptor Substrate-1
MPA:	Medical Products Agency
PKB/Akt:	Protein Kinase B
s.c:	Subcutaneous
SPR:	Surface Plasmon Resonance

Clinical

AE	Adverse Event
AESI	Adverse Event of Special Interest
AUC(0-10 h)	Area Under the plasma drug Concentration-time curve from time zero to 10 h
AUC-GIR(0-10 h)	Area Under the Glucose Infusion Rate-time curve from time zero to 10 h
BMI	Body Mass Index
CHMP	Committee for Medicinal Products for Human Use
Cmax	Maximum observed plasma Concentration
CI	Confidence Interval
CS	Clinically Significant
CV	Coefficient of Variation
EMA	European Medicines Agency
FAS	Full Analysis Set
GCP	Good Clinical Practice
GIR	Glucose Infusion Rate
GIRmax	Maximum GIR
HbA1c	Glycosylated Haemoglobin
IgE	Immunoglobulin E
IMP	Investigational Medicinal Product
LOESS	Local Regression
MAA	Marketing Authorisation Application
NCS	Not clinically significant
PD	Pharmacodynamics
PK	Pharmacokinetics
PPS	Per Protocol Set
PT	Preferred Term
Rechon Insulin	Rechon Insulin Human Soluble
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SUSAR	Suspected unexpected serious adverse event
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus

1. CHMP recommendation

Based on the review of the data and the applicant's response to the list of questions on quality, safety, efficacy, the application for Insulin Human Rechon, in the treatment of patients with diabetes mellitus who require insulin for the maintenance of glucose homeostasis is not approvable since a "major objection" was identified, which precludes a recommendation for marketing authorisation at the present time. The details of this major objection are provided in the List of outstanding issues (see section VI).

1.1. Questions to be posed to additional experts

No questions arise to additional experts.

1.2. Inspection issues

1.2.1. GMP inspection(s)

Conformance with EU GMP requirements is confirmed for all AS and DP manufacturing sites.

1.2.2. GCP inspection(s)

No request on GCP inspection arises upon clinical assessment. Please refer to section 2.3. regarding further information on GCP aspects.

1.3. Similarity with authorised orphan medicinal products

It is considered that Insulin Human Rechon is not similar to Amglida (glibenclamide; EU/3/15/1589) within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

2. Executive summary

2.1. About the product

The active substance of Insulin Human Rechon is human insulin. The primary activity of insulin is the regulation of glucose metabolism through several anabolic and anti-catabolic actions on a variety of different tissues. It lowers blood glucose by stimulating peripheral glucose uptake by skeletal muscle and fat, and by inhibiting hepatic glucose production. It also promotes conversion of glucose to its storage form, glycogen. Other functions of this hormone are to trigger amino acid uptake and conversion to protein in muscle cells and inhibit protein degradation. In addition, it stimulates triglyceride formation and inhibits release of free fatty acids from adipose tissue; and promotes lipoprotein lipase activity, which converts circulating lipoproteins to fatty acids.

Insulin Human Rechon is classified into the pharmacotherapeutic group "Insulins and analogues for injection, fast-acting", ATC code: A10AB01. Insulin Human Rechon is a soluble, fast-acting insulin that has been developed as a similar biological medicinal product (biosimilar) to the reference medicinal product Humulin® Regular (100 IU/mL). The reference medicinal product is Humulin ® Regular (100IU/mL) authorised in Sweden in 1987 (Eli Lilly Sweden AB, Marketing Authorisation No 10565) on the basis of a complete dossier. The Swedish Humulin® Regular is used as reference product. Data protection and market exclusivity of Humulin® Regular in Europe has expired.

Insulin Human Rechon is a sterile, clear, colourless, aqueous solution of human insulin presented in 3 ml cartridges. One ml contains 100 IU insulin human produced in *Escherichia coli* by recombinant DNA

technology. The recommended dose and route of administration of Rechon insulin are the same as for the reference product: *Determined by the physician, according to the requirement of the patient.*

The proposed indication, "*The treatment of patients with diabetes mellitus who require maintenance of glucose homeostasis*", is in line with the indication of the reference product.

2.2. The development programme/compliance with guidance/scientific advice

Two scientific advices were provided to this product: EMA provided scientific advice in June 2014 (EMEA/H/SA/2795/1/2014/III), whereas Sweden provided national scientific advice in September 2010 (Dnr 161:2010/511053). The procedures pertained questions regarding, quality, non-clinical and clinical development.

2.3. General comments on compliance with GMP, GLP, GCP

GMP

For the manufacturing site responsible for drug substance (DS) manufacturing, packaging and release, the Swedish Medical Products Agency has issued a GMP certificate.

EU importation and final batch release of the DS by the Qualified Person (QP) is the responsibility of the manufacturer.

For the drug product (DP) manufacturer, a GMP certificate issued by the Swedish MPA is presented along with a respective manufacturer's authorisation. The certificates refer to the manufacture and importation of human medicinal products.

GLP

The pivotal 28-day repeated-dose toxicity study by s.c. route in rats (including toxicokinetic profile, local tolerance and blood glucose profiles) performed with the biosimilar Insulin Human Rechon compared to the innovator product Humulin® Regular was conducted in compliance with the principles of GLP.

GCP

Both RCT-001 and RCT-004 clinical trials were in compliance with the protocol, regulatory requirements, good clinical practice (GCP) and the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association.

Study RCT-001 was conducted at one site in Sweden. No GCP inspections had been requested nor taken place by any regulatory authority during the conduct of the clinical trial and no inspections are planned or requested.

Study RCT-004 was conducted at 2 sites in Germany and 21 sites in Poland. No GCP inspections had been requested nor taken place by any regulatory authority during the conduct of the clinical trial and no inspections are planned or requested.

2.4. Type of application and other comments on the submitted dossier

2.4.1. Legal basis

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC, as amended – relating to applications for biosimilar medicinal products.

2.4.2. Biosimilarity

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: Humulin® Regular, 100 IU/ml, 3 ml cartridge
- Marketing authorisation holder: Eli Lilly and Company Ltd
- Date of authorisation: 31st March 1987
- Marketing authorisation granted by:

- Member State (EEA): Sweden

  - National procedure

- Marketing authorisation number: 10565

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

- Product name, strength, pharmaceutical form: Humulin® Regular, 100 IU/ml, 3 ml cartridge
- Marketing authorisation holder: Eli Lilly and Company Ltd
- Date of authorisation: 31st March 1987
- Marketing authorisation granted by:

- Member State (EEA): Sweden

  - National procedure

- Marketing authorisation number: 10565

■ Medicinal product which is or has been authorised in accordance with Union provisions in force and to which comparability tests and studies have been conducted:

- Product name, strength, pharmaceutical form: Humulin® Regular, 100 IU/ml, 3 ml cartridge
- Marketing authorisation holder: Eli Lilly and Company Ltd
- Date of authorisation: 31st March 1987
- Marketing authorisation granted by:

- Member State (EEA): Sweden

  - National procedure

- Marketing authorisation number(s): 10565

Rechon Insulin development programme has tested similarity between Insulin Human Rechon and Humulin® Regular reference product in line with current European Medicines Agency (EMA) guidelines on similar biological medicinal products:

- EMA (2015): Guideline on similar biological medicinal products (CHMP/437/04 Rev 1)
- EMA (2014): Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) (EMA/CHMP/BWP/247713/2012)
- EMA (2015) Guideline on similar biological medicinal products containing biotechnology derived proteins as active substance: non-clinical and clinical issues. EMEA/CHMP/BMWP/42832/2005 Rev1.
- EMA (2015) 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.

2.4.3. Information on paediatric requirements

Not applicable.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

The finished product Insulin Human Rechon is presented as 100 IU/ml solution for injection containing 100 IU/mL of recombinant insulin human as active substance.

Other ingredients are: glycerol, m-cresol, hydrochloric acid, sodium hydroxide and water for injection (WFI).

The product is available in 3 mL cartridges to be used only in conjunction with reusable insulin pen.

3.1.2. Active substance

3.1.2.1. General information

Recombinant human insulin is structurally identical to the native human insulin and composed of two peptide chains, A-chain containing 21 amino acid residues and B-chain with 30 amino acids. A- and B-chain are connected by two intermolecular disulphide bonds and the A-chain contains one additional intramolecular disulphide bond. Human Insulin is provided as crystals consisting of insulin hexamers.

3.1.2.2. Manufacture, process controls and characterisation

Description of manufacturing process and process controls

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The AS is produced by **recombinant technology** in *Escherichia coli*. The AS upstream manufacturing process is a conventional process starting with the inoculum build up based on the WCB, and comprising several stages of culture expansion from shaking flask up to a final fermenter volume in batch mode. Following induction, cells are harvested. The expressed primary product, single chain precursor (SCP) protein, is stored in inclusion bodies (IB). At harvest, the cells are recovered and lysed. Inclusion bodies are solubilised and the solubilised protein is separated. The obtained dissolved SCP protein is subjected to step-wise purification during the downstream processing. Purified fractions are combined and human Insulin is crystallized and recrystallized to yield the final human Insulin AS. The recrystallized human Insulin is dried and blended to obtain microcrystalline human Insulin AS.

An acceptable flow chart containing information on the main process steps including material flow, process parameters and in-process controls and their acceptance criteria, and critical steps is provided for the manufacturing process. The description of the manufacturing process is adequately described. Information on classification of CPPs and non-CPPs is provided. In-process controls (IPCs) in downstream process are described. The nature of product-related substances has been sufficiently elucidated. Microbial quality is sufficiently controlled.

Re-processing of process materials is not foreseen in the manufacture of the AS.

Control of materials

In general, the generation of the cell substrate is well described. The cloning strategy of the expression plasmid has been sufficiently described. An acceptable schematic of the final expression plasmid is included in the dossier and its features have been adequately described. Sufficient information on the host cell line E. coli strain has been provided.

A two-tiered banking system consisting of a master cell bank (MCB) and a working cell bank (WCB) has been established … A storage system for the MCB and WCB is in place. The generation of MCB/WCB is adequately described. Information on the establishment and control of future WCBs is presented.

The characterization testing program of MCB and WCB is considered sufficient to identify all relevant phenotypic and genotypic characteristics. Cell bank stability has been sufficiently demonstrated for storage for the MCB. The WCB will be tested, which is endorsed.

End of production cell banks (EPCBs) and cells beyond-production have been produced at commercial manufacturing scale and according to the commercial manufacturing process. Overall, genetic stability of EPCBs at the limit of in-vitro cell age (LIVCA) is considered confirmed.

The raw materials used for cell culture and purification are listed in the dossier. sufficient specifications are included in the dossier. No raw materials derived from animal sources are used in the process. Adequate information on the qualitative composition of cell bank culture media and on resin columns and filters used during the manufacture of human Insulin AS have been provided.

Control of critical steps and intermediates

Sufficient information on control of critical steps and intermediates is provided. Critical process parameters (CPPs) along with their control ranges are stated. CPPs along with respective control ranges for each manufacturing step are presented and the proposed control ranges are justified. IPCs and their corresponding acceptance criteria as defined for the AS manufacturing process are listed in 3.2.S.2.4. A comprehensive summary of CQAs as defined for Human Insulin AS are presented in legally binding section 3.2.S.2.4.

No process intermediates are reported. Validated maximum holding times between different manufacturing steps are stated.

Process validation and/or evaluation

Process validation of the AS manufacturing process follows a process validation campaign.

Three consecutive AS batches manufactured at the intended commercial scale and the intended commercial manufacturing site have been included in the latest process validation campaign. The critical process parameters and in-process controls at each stage have been monitored as per the process validation protocol. All evaluated critical process parameters for the upstream/downstream process were found to be within the pre-defined ranges. Key and non-critical process parameters were also verified to be within the specified ranges meeting the production requirements. Each lot met in-process limits, demonstrating consistent operations, AS batches complied with the current AS release specification. In conclusion, the consistency and reproducibility of the Human Insulin AS manufacturing process has been adequately confirmed, supporting the proposed control strategy.

To demonstrate the process clearance capability of process-related and product-related impurities, depletion has been evaluated by monitoring these impurities during the manufacture after each purification step. Residual levels in AS consistently met pre-defined acceptance criteria.

Sufficient information is provided about analytical methods used during validation of process clearance capability of impurities.

Shipping conditions have been adequately described, supported by respective shipping validation data.

Manufacturing process development

The comparability assessment was performed, taking into consideration ICH Q5E. Overall, analytical comparability of materials prior and-post-change has been sufficiently demonstrated. Representativeness of materials used for evaluation of biosimilarity has been adequately verified as well. Overall, the developmental history of the Human Insulin AS manufacturing process is sufficiently depicted.

Moreover, an appropriate control strategy/action for each CPP has been developed.

As regards in-process control tests, the proposed acceptance criteria are based on prior knowledge and data from historical commercial-scale batches. As supportive, analytical IPC batch data from representative AS batches have been provided.

Critical process steps in the manufacture of Human Insulin AS have been defined. Critical steps and their respective controls are adequately summarized in dossier section 3.2.S.2.4.

Critical quality attributes (CQAs) of Human insulin AS were determined by a risk assessment to identify the criticality of the impacts of variation of product attributes on the safety and/or efficacy for the patient. A comprehensive summary of CQAs as defined for Human Insulin AS should be included in legally binding section 3.2.S.2.4.

Overall, the control strategy has been set up appropriately in line with ICH Q11 guidance and has been sufficiently elaborated and justified, in particular with respect to criticality classification of process steps, process parameters and process controls.

Characterisation

The characterisation studies were executed using different AS batches. It has been clarified that the AS batches used for characterisation studies can be considered to be representative for the current commercial process and the clinical material.

The primary and secondary structure of the AS were studied in comparison to literature data.

The product-related impurity profile has been shown that the in-house impurity method included in the AS specification is sufficiently specific to resolve the specified and unspecified impurities from the main peak and to resolve all impurities from each other. To further evaluate the AS impurity profile and to build up a sound knowledge data base, stress studies in line with ICH requirements were performed. Based on that, degradation pathways have been investigated and the stability indicating power of analytical methods could be sufficiently demonstrated.

In-depth analysis of the specific product-related impurity profile has been performed. Based on these investigations and based on data set, acceptable AS acceptance criteria for product-related impurities as analysed are proposed for the AS release and shelf-life specification.

3.1.2.3. Specification, analytical procedures, reference standards, batch analysis, and container closure

Active Substance Specifications

For the active substance recombinant human insulin, a specification has been provided comprising testing of appearance, identity by HPLC, determination of assay including human insulin and A21-desamido insulin by HPLC, and determination of purity and related substances and impurities.

As regards Ph. Eur. monograph for recombinant human insulin (0838), the pharmacopoeial test parameter and overarching test procedures are included in the proposed AS specification, however, the test methods are mostly in-house methods. A clear reference to identify the analytical methods applied for and linking AS specification, method description and method validation documents is included in the AS specification.

Analytical procedures and reference standards

Most of the analytical procedures applied for control of the AS are in-house methods for which acceptable descriptions have been provided. Validation of the proposed analytical procedures included in the AS specification is considered acceptable.

Batch data are presented. All batches have been used in manufacturing process (FP/AS) validation activities and for clinical phase III study. All data are found complying with the acceptance criteria being in place at the time of analysis. In addition, AS batch release data encompassing also the product-related impurity profile. Also these data were found complying with the acceptance criteria being in place at the time of analysis.

Based on the presented data, acceptance criteria for the product-related impurity profile included in the AS specification have been reasonably revised. As regards TAMC and TYMC, the proposed acceptance criteria are considered adequate.

In the human insulin MA applied for, Ph. Eur. Human insulin CRS is used as primary standard. For future establishment of human insulin working standard, an adequate protocol of the qualification program has been provided.

Container closure

Acceptable specifications are provided for the CCS and compliance with Ph. Eur. Monograph 3.2.2 has been demonstrated.

3.1.2.4. Stability

Stability data are provided. In addition, long-term (–20°C ±5 °C) and accelerated (5°C± 3°C) stability data are available with the proposed commercial AS manufacturing process. Stability batches are packed into containers of the identical material but smaller volume.

AS release and shelf-life specification is provided in section 3.2.S.4.1. Tighter limits have been defined for the release than for the shelf-life of the active substance which is considered acceptable and in line with ICHQ6B guideline. The specified product related substances and impurity profile is addressed in the shelf-life specification. With the provided updated AS stability data including the impurity profile, the AS batches are shown complying with the AS specification even at the end of shelf life.

The provided stability data covering long term storage up to 36 months and accelerated storage up to 6 months were found complying with the acceptance criteria. The provided data support the AS shelf-life claim of 36 months at −20°C ±5 °C storage conditions.

Forced degradation studies have been performed.

3.1.3. Finished Medicinal Product

3.1.3.1. Description of the product and pharmaceutical development

Insulin Human Rechon 100 IU/ml, solution for injection in cartridge, is a solution for subcutaneous injection. The product is filled in 3 ml cartridges, with a lined seal and a rubber plunger. The extractable volume is 3 ml/cartridge. The formulation contains glycerol, m-cresol, hydrochloric acid, sodium hydroxide and water for injection.

Pharmaceutical development

The composition of the FP is similar to the composition of the reference product Humulin Regular. The same FP formulation has been used throughout development including clinical studies.

Relevant attributes of the FP have been defined. Controls for these attributes have been established in the manufacturing process. A quality target product profile (QTPP) for the finished product has been provided. CPPs have been defined based on a risk assessment and respective controls listed together with their ranges/acceptance criteria.

The container closure system of the FP is similar to that of the reference product. For dosing, a pen is used. Design verification, dose accuracy and usability were adequately analysed. Container closure integrity and compatibility of the FP with the cartridge was demonstrated by in-use stability studies. Evaluation of extractables/leachables of the container closure system has been performed. The applicant has provided summarized data from extractable studies. Also, the applicant has provided summarized results from leachable studies performed with the three expired FP batches. No relevant amount of extractables and leachables has been found in the studies. The applicant's conclusion that the chosen CCS can be considered suitable for the intended purpose is supported based on the provided data.

Nevertheless, the applicant plans to perform a leachable study employing stressed FP, which is endorsed. The (summarized) results of this study should be provided, when available, together with the final E/L study report [**REC**].

3.1.3.2. Manufacture of the product and process controls

The FP manufacturing process is comprehensively described and comprises five steps: sterilisation of equipment and primary packaging components, compounding, sterile filtration and final dilution, filling and packaging. A flow-diagram is provided indicating critical process parameter (CPP) and in process controls (IPC). The critical process steps have been identified in a risk assessment. The process description details the manufacturing steps and process parameter with their respective limits or

ranges. The acceptance criteria for the CPP have been justified based on process validation data. Sterile filtration is. Bioburden is determined.

Process hold times and the maximal process time have been indicated, as requested.

The process validation (PV) has been performed. It has been clarified that the PV has not been performed in a retrospective manner but prospectively planned. Nevertheless, a PV campaign manufactured 'consecutively' in biannual intervals is somewhat unusual though considered acceptable. Process re-validation is committed to be performed before commercial distribution. In general, the approach is still unusual as it is relying on very few and old process validation data. Nevertheless, provided, that the re-validation comprising the first three commercial scale FP batches is successfully performed and respective data are submitted for assessment before commercial distribution and fully support a validated state of the manufacturing process, the strategy is considered acceptable. [**REC**]

Product specification, analytical procedures, batch analysis

The FP release and stability specifications have been updated.

The shelf-life specification for the FP contains a reduced list of parameter, which is considered adequate. The FP is labelled in units. The units are calculated based on the measured protein amount by HPLC using a conversion factor of 0.0347 mg of human insulin which is equivalent to 1 IU of human insulin.

Generally, most acceptance criteria set in the FP specification are considered acceptable. In general, the database on which the criteria are based is extremely limited.

Analytical procedures and reference standards
In-house methods have been indicated in the FP specification and linked with a unique identifier while compendial methods reference the respective Ph. Eur. monographs. The descriptions of the analytical methods have been expanded as requested to contain more details on the procedures employed. For some parameters required to be tested by Ph. Eur. 854, alternative methods have been used interchangeability of these in-house methods with the compendial methods has been demonstrated.

A table has been provided indicating the reference standards (RS) for the respective methods used. Certificates of origin and information leaflets have been provided for the compendial reference standards. The currently established Insulin human RS has been identified including the batch number and manufacturing date.

Validation of analytical methods
All compendial methods have been qualified according to the general requirements in Ph. Eur. Validation reports have been provided for the non-compendial methods and are considered acceptable.

Batch analysis
Batch release data of commercial finished product batches have been presented. All results were found to be in compliance with the release specification at time of release, though the database is very scarce.

Characterisation of impurities
The characterization of product-related substances and impurities has been expanded as requested. Nevertheless, no negative impact is expected due to adequate control of these impurities in the FP specification. Further, the impurity profiles of the active substance and the finished product has been compared and impurities and/or substances unique to the finished product have been indicated and their impact on the FP quality is discussed.

Elemental impurities (EI) have been discussed in accordance with ICH Q3D requirements. As the results were well below limits and met the control threshold of 30% of PDE, no routine analysis has been established. This is considered acceptable. A risk assessment for nitrosamines has been performed and provided, evaluating each step of the manufacturing process. No risk for the contamination of the finished product with nitrosamines was identified. This is considered adequate.

Container closure

In general, adequate information has been provided on the container closure system of the finished product. Manufacturers have been identified and adequate specifications on the cartridge, lined seal and plunger provided. Compliance with Ph. Eur. 3.2.9 has been confirmed. The glass of the cartridges is compliant with Ph. Eur. 3.2.1.

3.1.3.3. Stability of the product

The proposed shelf-life when stored at 5°C ± 3°C and an in-use shelf life at 25°C ± 2°C is endorsed.

Stability studies on the FP have been conducted in accordance to ICH requirements. The same analytical procedures have been employed for FP release and stability testing. The stability data comply with the acceptance criteria at long-term storage conditions at 5±3°C. All batches showed increases in the amounts of impurities/related substances and a slight decrease in insulin content. Nevertheless, the data still remain within specification limits.

3.1.3.4. Biosimilarity

To demonstrate analytical comparability between the test product (Insulin Human Rechon) and the reference product, Humulin R, two head-to head studies have been. Additionally, an extensive comparative forced degradation study has been performed.

The studies have been performed using a low number of FP batches. It has been clarified, that the FP batches manufactured used for the analytical similarity study, can indeed be considered representative. Also, the AS batches used for manufacture of these FP batches can be considered representative for the commercial AS manufacturing process,
A number of batches of the reference medicinal product Humulin R have been included in the comparability study, too. These Humulin R batches have an EEA "country of origin", i.e. they are medicinal product authorised in the EEA.

Both the test product and the reference product have been isolated using the same procedure in order to obtain the pure AS for further analytical investigation.

At the time of the first comparability study, the test batches were older than the reference batches which had a closely related age.. Roughly, the test batches were "older" than the reference batches and two of the test batches were already expired.

An extensive analytical program has been explored for the analytical comparability study. Overall, the chosen panel of analytical methods seem suitable for the intended use and including also orthogonal analytical methods appropriate to detect differences between the test and the reference, if any.

No specific statistic approach has been applied; this does not compromise the assessment of the provided comparability data. However, no comparability/similarity acceptance criteria based on investigation of the reference medicinal product batches have been derived and pre-defined. Qualitative and quantitative analytical data have been solely visually compared or evaluated by simple comparison of numbers. Since no borderline or critical results have been detected in this study, this may be acceptable in this case.

Table 1: Biosimilarity exercise, comparability data

Attribute	Analytical Method	Comparability outcome
Amino acid composition	Amino acid analysis	Comparable
Assessment of N- and C-termini	Edman degradation	Comparable
Assessment of N- and C-termini	LC-MS sequencing	Comparable
Amino acid sequence	Peptide mapping/LC-MS	Comparable
Molecular weight	Molecular Mass (intact)	Comparable
Molecular weight	Molecular Mass (reduced)	Comparable
Post-translational modifications	Peptide mapping/LC-MS	Comparable
Disulfide bridges	LC/ES-MS	Comparable
Charge variants	icIEF (capillary isoelectric focussing)	Comparable
Size variants/protein aggregation	SEC (size exclusion)	Comparable
Size variants/protein aggregation	Ultracentrifugation	Comparable
UV profile	HPLC-UV	Comparable
Protein concentration	Amino acid analysis	Comparable
Optical density	UV spectrocopy	Comparable
Secondary structure	A number of spectroscopic techniques	Comparable
	Thermal stability	Comparable

Structural comparability of insulin active substance samples isolated from the Insulin Human Rechon FP and the reference product Humulin R was studied using physico-chemical state-of-the-art analytical methods. Similarity between the test and the reference product with regard to primary, secondary and tertiary structure, molecular mass and size distribution is postulated and can be considered overall justified by the provided data. Comparative peptide maps of intact peptide, A-chain and B-chain as well as respective MS data are only available for very early batches. Differences between the test and the reference product are observed with regard to deamidation and high molecular weight substances. Charge variants as determined in the Insulin Human Rechon batches and in Humulin R batches are most probably assigned to deamidated variants. Higher deamidation levels in the test product are also

detectable in the RP HPLC-UV profiles. Deamidation in the A-chain is found similar both in the test and in the reference product, while deamidation in the B-chain is higher in the insulin Rechon batches.

In the forced degradation study comparing one Insulin Human Rechon FP batch against one Humulin R batch, stress, as impacted by variation of pH, temperature, oxidation/reduction, light, freeze/thaw and mechanical stress has been investigated. Mainly, the investigated stressors induce comparable elevated levels of deamidation and oxidation as well as increased levels of HMWS and LMWS.

An additional analytical comparability study has been performed to further investigate HMWS formation and deamidation as a time related process.
Based on the results, linear dependency of batch age and deamidation in the B-chain is postulated that is found similarly in the test product and the reference products. Higher HMWS levels are observed in the test batches only and this is not found to be related to the sample age. Instead, this is considered a real difference between the test and the reference product.

While extensive analytical comparability data have been provided analysing the insulin active substance isolated from FP test and FP reference batches, some data have been provided comparing the test FP and the reference finished product directly.

Some slight differences between the test and the reference product are observed.
However, based on the totality of analytical data provided, there is no doubt that insulin Human Rechon finished product manufactured most recently in 2019 is comparable to Humulin R reference product.

3.1.3.5. Post approval change management protocol(s)

Not applicable

3.1.3.6. Adventitious agents

Insulin Human Rechon is produced in *Escherichia coli*. No human or animal derived materials are used in the manufacturing process. Therefore, the risk of viral and TSE contamination of the product is considered negligible.

3.1.3.7. GMO

Not applicable

3.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

The application for Insulin Human Rechon containing recombinant human insulin as active substance is approvable from a quality point of view as the outstanding issues have been adequately addressed by the applicant. Suitability of the chosen primary packaging for the finished product has been demonstrated by respective extractable/leachable (E/L) data. The final E/L study report including the (summarized) results of the leachable study with stressed FP samples should be provided when available. **[REC]**

The active substance manufacturing process narrative is generally sufficiently detailed. Recombinant human insulin is expressed as single chain precursor protein in inclusion bodies in E.coli cells. The downstream process enzymatically converts the precursor protein to insulin that is subsequently

chromatographically purified. Based on the AS process validation campaign, the proposed commercial AS process is in a validated state.

In- depth characterisation and evaluation of the specific product-related impurity profile of the active substance has been performed using adequate analytical procedures including MS techniques. Based on these investigations and based on data set of numerous AS batches, reasonable acceptance criteria have been proposed for the AS specification.

In general, the quality of the FP is considered acceptable. The process validation (PV) has been performed in 2020, employing non-recently manufactured batches. Although considered acceptable, re-validation of the manufacturing process will be performed and data provided. [**REC**]

For the biosimilar Insulin human Rechon an extensive analytical similarity study has been conducted. Based on the totality of analytical data, the claim of analytical similarity between the Insulin human Rechon test product and the reference product Humulin R is supported.

3.2. Non-clinical aspects

3.2.1. Introduction

Insulin Human Rechon is a fast-acting insulin that has been developed as a similar biological medicinal product (biosimilar) to the reference medicinal product Humulin® Regular. Insulin Human Rechon is indicated for the treatment of patients with diabetes mellitus who require insulin for the maintenance of glucose homeostasis. The primary activity of insulin is the regulation of glucose metabolism through several anabolic and anti-catabolic actions on a variety of different tissues. It lowers blood glucose by stimulating peripheral glucose uptake by skeletal muscle and fat, and by inhibiting hepatic glucose production. It also promotes conversion of glucose to its storage form, glycogen. Other functions of this hormone are to trigger amino acid uptake and conversion to protein in muscle cells and inhibit protein degradation. In addition, it stimulates triglyceride formation and inhibits release of free fatty acids from adipose tissue; and promotes lipoprotein lipase activity, which converts circulating lipoproteins to fatty acids.

Insulin Human Rechon is a sterile, clear, colourless, aqueous solution of human insulin presented in 3 ml cartridges. One ml contains 100 IU insulin human produced in Escherichia coli by recombinant DNA technology. Insulin Human Rechon is identical to the naturally occurring human insulin. Its pharmacotherapeutic group is "Insulins and analogues for injection, fast-acting", whereas the corresponding ATC code for this product is "A10AB01".

The reference medicinal product is Humulin ® Regular (100IU/mL) authorised in Sweden in 1987 (Eli Lilly Sweden AB, Marketing Authorisation No 10565) on the basis of a complete dossier. Data protection and market exclusivity of Humulin® Regular in Europe has expired. The claimed changes to the reference product are changes in the raw material(s) and in the manufacturing process(es).

3.2.2. Pharmacology

3.2.2.1. Primary pharmacodynamic studies

The applicant submitted comparative in vitro PD studies in which biosimilarity between Insulin Human Rechon and its comparator Humulin® Regular from Eli Lilly were studied in receptor binding assays as well as in cellular in vitro assays. The latter comprise assays in insulin-sensitive rat adipocytes in which autophosphorylation of the insulin receptor was indirectly studied by examining the extent of phosphorylation of downstream signalling kinases, and by studying metabolic activation of the adipocytes by the different insulin products. Specifically, metabolic activation was examined by

measuring glucose uptake, lipogenesis and inhibition of lipolysis of the rat adipocytes when the different insulin products were added to the cell cultures. This experimental setup is considered sufficient for the non-clinical development of an insulin biosimilar product.

In the receptor binding study, data were gathered in six consecutive experiments, whereas in the cellular in vitro studies treatments were carried out mostly in triplicates (and only occasionally in duplicates) for up to 3 consecutive experimental runs. Importantly, three batches of the investigational Insulin Human Rechon product and seven different batches of the insulin comparator Humulin® Regular were used for that purpose. This experimental strategy is also considered to suffice for the development of an insulin biosimilar product; no concerns were identified on this aspect. However, note that the comparability of the three Insulin Human Rechon batches used in the biocomparability studies with the Insulin Rechon product proposed for marketing is uncertain. This is the matter of a concern in the quality assessment of this procedure.

<u>In vitro bioassays for receptor binding (Study 84210811):</u>

As mentioned in the EMA guideline EMEA/CHMP/BMWP/32775/2005_Rev. 1, in vitro receptor binding assays are one of the two paramount pillars of a non-clinical PD biosimilarity assessment of an insulin biosimilar product. In this submission, the applicant conducted a panel of in vitro receptor binding assays in which binding of Insulin Human Rechon and its comparator insulin product Humulin® Regular onto the two isoforms of the insulin receptor (IR isoform A and isoform B) and onto the IGF-1 receptor was examined.

The binding of the investigational Insulin Human Rechon product to the immobilised receptors on the proved to be well reproducible within the tested concentration range in all six replicates of the particular treatments. Importantly, the binding of Insulin Human Rechon to the immobilised receptors also proved to be highly similar to the binding of Humulin® Regular batches. This shows that in the in vitro receptor binding Study 84210811 the applicant sufficiently demonstrated biosimilarity of Insulin Human Rechon and its comparator insulin product Humulin® Regular to the relevant receptors (the two IR isoforms A and B and IGFR-1).

The following Figure shows the binding of Insulin Human Rechon and Humulin® Regular to the IR long, short and IGFR-1 receptor:

Figure 1: Concentration-dependent RU readouts for Humulin® Regular and Insulin Human Rechon batches for Insulin Receptor (IR) long.

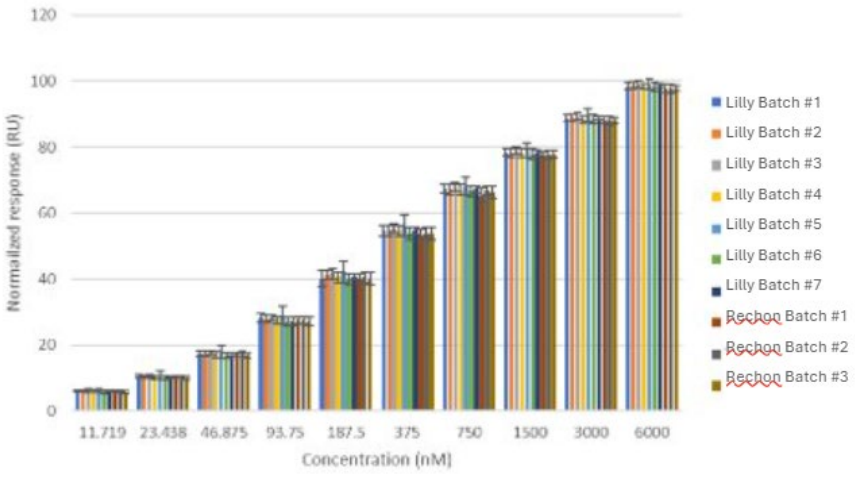


Figure 2: Concentration-dependent RU readouts for Humulin® Regular and Insulin Human Rechon batches for IR short.

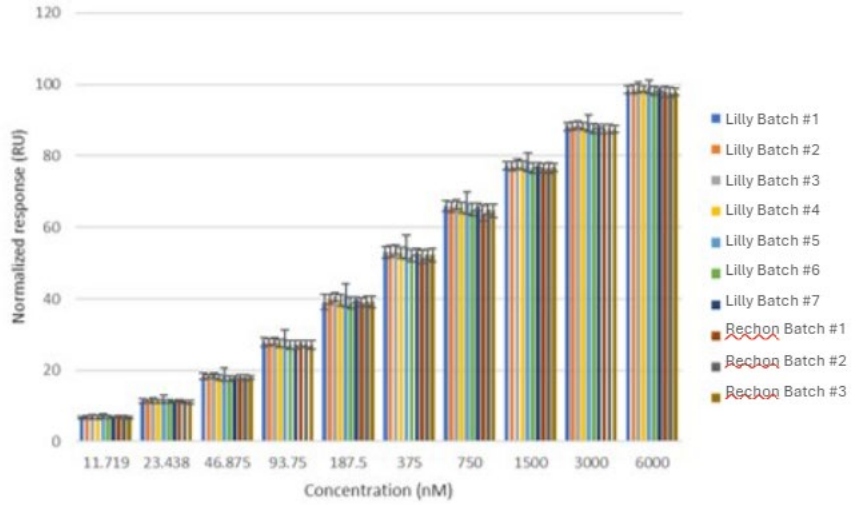
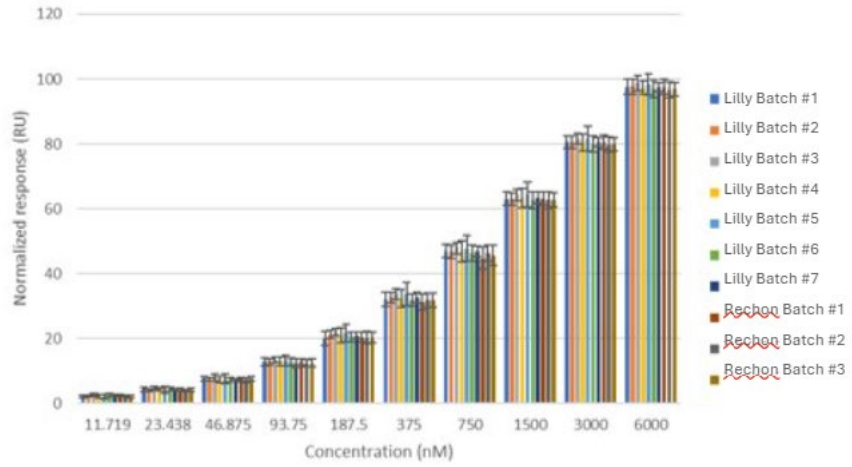


Figure 3: Concentration-dependent RU readouts for Humulin® Regular and Insulin Human Rechon batches for insulin receptor IGFR-1.



In vitro biological activity:

In the preliminary Study 2021-11-29-Rechon and in the pivotal Study 2021-10-28-Rechon, the applicant conducted different cellular biosimilarity assays in which metabolic activation of insulin-sensitive cells Specifically, metabolic activation by the different insulin products was studied in terms of glucose uptake, lipogenesis and lipolysis inhibition,

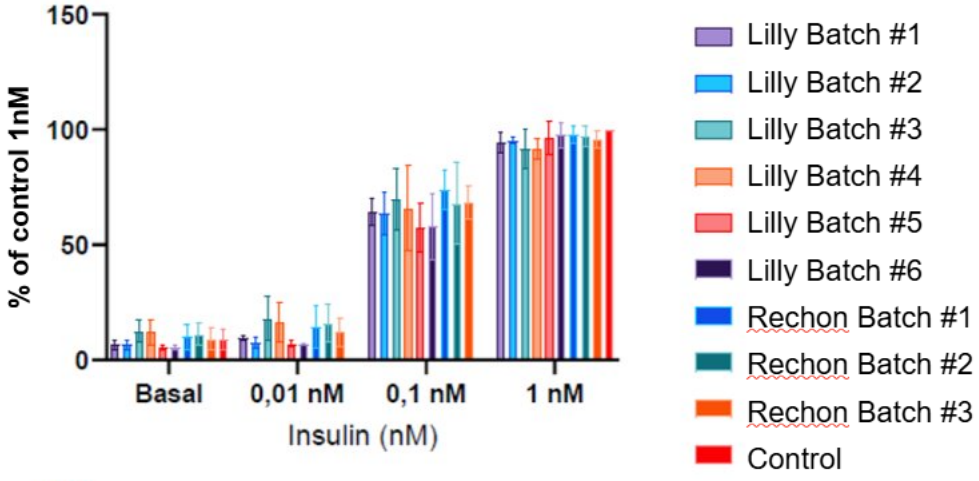
These investigations were conducted with the Insulin Human Rechon batches and with the Humulin® Regular batches. Most experiments were conducted in triplicates in the particular experimental run, only some of the experiments were conducted in duplicates. Of note, a control was used in each of the separate experiments at 100 IE/ml, and all experimental results gathered by incubations with Insulin Human Rechon or Humulin® Regular were normalised to these control measurements.

This in vitro biosimilarity programme is in accordance with the recommendations given in the EMA guideline EMEA/CHMP/BMWP/32775/2005_Rev. 1 on the non-clinical and clinical development of biosimilar insulin products.

In the following, the results of the pivotal in vitro biosimilarity Study 2021-10-28-Rechon are described:

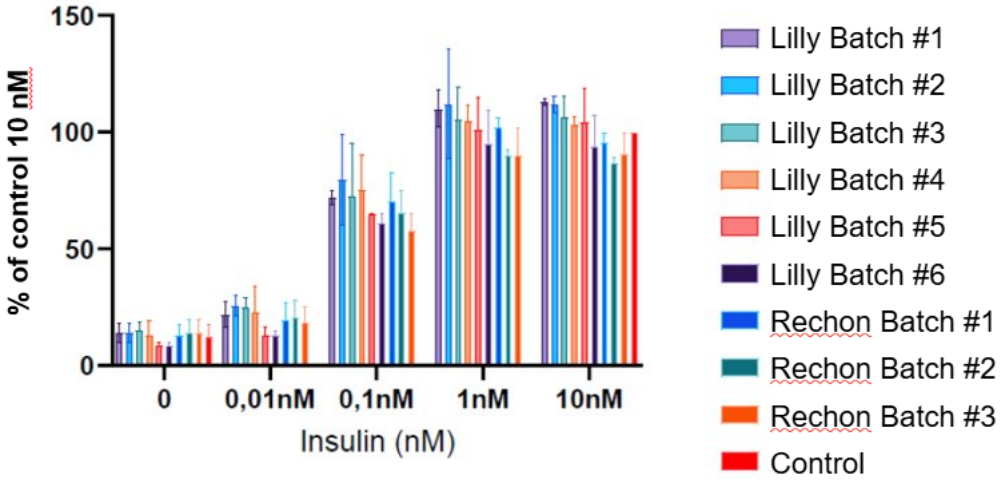
Glucose uptake: In the first fraction of Study 2021-10-28-Rechon, the applicant investigated whether the investigational insulin Rechon and the comparator insulin product Humulin® Regular lead to a similar uptake of glucose in insulin-sensitive cells. In this experiment, no relevant differences in cellular glucose uptake between the tested Insulin Human Rechon and Humulin® Regular batches were noted at 0.1 and 1 nM (see Figure below). This suggests biosimilarity of both insulin products in terms of stimulating cellular glucose uptake.

Figure 4: Glucose uptake
In each experiment, all samples were analysed in triplicates and the mean value from each experiment was used for the summary graphs. In each experiment, a control and the effect of the other insulin batches was related to the control. Data are presented as % of control response (n=3).



The results of this experiments (see Figure below) demonstrate that all batches of the two tested insulin products stimulated lipogenesis. Largely, the increase in lipogenesis was similar between the tested Humulin® Regular and the Insulin Human Rechon batches. However, it is apparent that some Humulin® Regular batches produced a clearly higher extent of lipogenesis than the tested Insulin Human Rechon batches. For example, the Humulin® Regular batches produced a higher extent of lipogenesis at 10 nM than the batches of Insulin Human Rechon did. However, no concern was raised on this aspect, as other Humulin® Regular batches led to a comparable extent of lipogenesis than the Insulin Human Rechon batches. Furthermore, the studied Insulin Human Rechon batches at 10 nM led to a comparable extent of lipogenesis in comparison to the ® control samples. Finally, at 1 nM and especially 0.1 nM, the extent of lipogenesis between the Insulin Human Rechon and Humulin® Regular batches were more alike as in the 10 nM experiments. Considering these aspect, it is apparent that Insulin Human Rechon produced a similar extent of lipogenesis than the analysed Humulin® Regular batches. Therefore, no concern was raised.

Figure 5: Lipogenesis
In each experiment a 10 nM control is used and the effect of the other insulin batches was related to the response of control. Data are presented as % control response (n=3).



The results of this experiment demonstrate that the addition of isoprenaline indeed stimulated lipolysis, as measured by a clearly increased release of glycerol from the cells. Concomitant addition of the two different insulin products clearly hampered this effect. However, the extent of inhibited lipolysis by addition of the two different insulin products varied among the experiments conducted in this study, whereby in experiment 2 the inhibitory effect was smallest. Nonetheless, apart from this inter-experimental variance, the extent of inhibited lipolysis was identical between the tested Insulin Human Rechon and Humulin® Regular batches. Of note, the extent of inhibited lipolysis by addition of Insulin Human Rechon and Humulin® Regular was similar as in the samples to which control sample insulin was added.

To conclude, this experiment demonstrated that insulin Rechon similarly inhibited lipolysis as the comparator insulin substance Humulin® Regular.

The analyses of phosphorylated IRS1 demonstrated that both insulin products and also the control increased phosphorylation of IRS1 at 1 nM, whereas at the lower insulin concentrations phosphorylation was presumably not statistically significant. Standard deviations in the different insulin treatments at 1 nM were comparatively large in this experiment. However, both Insulin Human Rechon and Humulin® Regular insulin batches at 1 nM produced a similar extent of IRS1 phosphorylation. Similarly, the phosphorylation proved to be very similar at 0.1 and 1 nM of the different insulin products, also demonstrating similarity between the Insulin Human Rechon and Humulin® Regular insulin batches Of note, the phosphorylation was clearly elevated above the negative control samples already at 0.1 nM of the different insulin products. Finally, phosphorylation was also clearly elevated at 0.1 and 1 nM of the two insulin products relative to the control samples, whereby no relevant differences between the Insulin Human Rechon and Humulin® Regular insulin batches were apparent.

To conclude, phosphorylation of the downstream signalling kinases of the insulin receptor was clearly demonstrated when both Insulin Human Rechon and Humulin® Regular insulin were added to insulin-sensitive rat adipocytes. Importantly, no clear differences in phosphorylation were observed between the analysed batches of Insulin Human Rechon and its comparator insulin product Humulin® Regular.

Therefore, biosimilarity in terms of insulin autophosphorylation was demonstrated in Study 2021-10-28-Rechon.

Apart from theses primary pharmacodynamics studies, no secondary pharmacodynamics, safety pharmacology or pharmacodynamic drug interactions studies were submitted.

Altogether, the submitted non-clinical PD programme is considered to be sufficiently extensive for examining the in vitro biosimilarity of an insulin product.

3.2.2.2. Secondary pharmacodynamic studies

No studies were submitted.

This is acceptable and in accordance with relevant guidance on the non-clinical development of an insulin biosimilar product (EMEA/CHMP/BMWP/32775/2005_Rev. 1).

3.2.2.3. Safety pharmacology programme

No studies were submitted.

This is acceptable and in accordance with relevant guidance on the non-clinical development of an insulin biosimilar product (EMEA/CHMP/BMWP/32775/2005_Rev. 1).

3.2.2.4. Pharmacodynamic drug interactions

No studies were submitted.

This is acceptable and in accordance with relevant guidance on the non-clinical development of an insulin biosimilar product (EMEA/CHMP/BMWP/32775/2005_Rev. 1).

3.2.3. Pharmacokinetics

The applicant did not submit studies on the pharmacokinetics of Insulin Human Rechon.

3.2.4. Toxicology

3.2.4.1. Single dose toxicity

Single-dose toxicity studies were not conducted in support of the MAA of Insulin Human Rechon which complies with the recommendations laid down in the EMA "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.

3.2.4.2. Repeat dose toxicity

A comparative GLP-compliant **4-week repeat-dose toxicity study** was performed in rats (Study 8241142). The test articles Insulin Human Rechon and another Insulin formulation as well as the reference medicinal products Humulin Regular were s.c. administered daily at doses of 1.2 and 2.4

IU/kg/day. The dose levels were chosen based on the clinical doses (low dose) as well as on the result of the 1-week dose range finding study in rats (high dose). A full toxicological evaluation was performed after the dosing period. No test-article related observations were observed.Inspection of the red cell parameters, however, revealed that all values were within the historical control range and are therefore not considered adverse. Also, injection sites in some cases exhibited dermatitis and fasciitis, which is not unexpected after s.c. administration of the test articles. Toxicokinetic analysis revealed overall comparable exposure among all test articles. However, inter-individual values varied considerably so that statistical evaluation was hampered also due to small group sizes per time-point analyzed. Exposure was dose-dependent albeit not dose-proportional for all test products and C_{max} almost consistently occurred between 0.25 and 2.00 hours post-dose. Regarding gender differences a generally higher exposure was observed in females as compared to males at the end of the administration phase.

3.2.4.3. Genotoxicity

No studies on the **genotoxicity** of Insulin Human Rechon have been performed which is acceptable for a biosimilar application.

3.2.4.4. Carcinogenicity

No studies on the **carcinogenicity** of Insulin Human Rechon have been performed which is acceptable for a biosimilar application.

3.2.4.5. Reproductive and developmental toxicity

Reproductive and developmental toxicity has not been evaluated for Insulin Human Rechon.

3.2.4.6. Toxicokinetic data

Please refer to section 3.2.4.2

3.2.4.7. Tolerance

No dedicated **local tolerance** studies have been conducted in support of the MAA of Insulin Human Rechon but local tolerance has been evaluated in the scope of the 4-week RDT study in rats. The applicant's approach is acknowledged. No findings unexpected for s.c. administration of the test articles were made.

3.2.4.8. Other toxicity studies

With regard to **impurities** a comprehensive panel of tests was performed with the biosimilar candidate in comparison to the innovator product. In general, both products proved to be highly similar. The only identified difference was the higher deamidation of the biosimilar candidate as compared to the innovator. As the deviation is ≤ 2.0% and, thus, still complies with the Ph.Eur. for injectable insulin preparations no concerns arise. Other product- and process-related impurities are controlled according to the limits set in the specification.

3.2.5. Ecotoxicity/environmental risk assessment

The applicant provided a justification for not conducting **ERA** studies for Insulin Human Rechon. This justification is based on the fact that the active ingredient is a peptide consisting of natural amino acids

which exempts the applicant from providing ERA studies as laid down in the EMA "Guideline on the environmental risk assessment of medicinal products for human use" (EMEA/CHMP/SWP/4447/00).

3.2.6. Discussion on non-clinical aspects

Pharmacodynamics:

The following concerns were originally identified:

It was noted that the in vitro PD biosimilarity programme submitted in Module 4.2.1 was earliest started in 2018. However, as scientific advice was already sought earlier than that (2010 from the Swedish agency, and 2014 from EMA), it was assumed that also older PD biosimilarity studies were conducted. The applicant was therefore expected to submit all biosimilarity PD studies that were conducted with Insulin Human Rechon. In response to this concern, the applicant submitted all comparative in vitro PD studies that were conducted prior to 2018. These studies are method development reports that support the pivotal study reports originally submitted for MAA. No concerns were identified in these reports.

Considering the cellular in vitro biosimilarity experiments, no justification was originally provided why primary rat adipocytes were used, but not human insulin-sensitive cells. The applicant was therefore expected to justify the choice of primary rat adipocytes as cell line for the in vitro metabolism and signal transduction biosimilarity assays, and to discuss why no human cell line was used for that purpose. The applicant responded and adequately justified the use of primary rat adipocytes as robust insulin-responsive cells by summarising their long use in literature for that purpose, the limited availability of primary human cells, and the smaller dynamic range of the insulin response in primary adipocytes isolated from human compared to rat adipose tissue. This concern was therefore resolved.

Additionally, the following concerns were identified in the main study 2021-10-28:

To show capability of insulin presentations to activate insulin receptors (IR-A, IR-B, IGF1-R), phosphorylation of signalling intermediates (Insulin Receptor Substrate-1 [IRS-1], Protein Kinase B [PKB/Akt] and Akt Substrate [AS160]) was determined. The applicant has partly 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) which recommends direct receptor stimulation capability determination by measuring autophosphorylation of IR-A and IR-B, respectively. The experiment was not designed in the way that it would be clear whether phosphorylation signal arises from pIR-A or pIR-B. Therefore, the applicant was asked to explain whether this indirect approach via determination of phosphorylation of signalling intermediates (IRS-1, PKB/akt, AS160) fulfils this criterion. As explained by the applicant, IR-B is the most dominant IR isoform in adipocytes which was used as an in vitro cell-based model in the experiments conducted. Thus, determination of intracellular phosphorylation of key targets IRS-1 (immediate downstream target of IR), PKB/Akt and AS160 (Akt substrate of 160kDa) after insulin induced auto-phosphorylation of IR receptors is considered sufficiently justified.

Glucose uptake, lipogenesis, signalling assays: A relatively small number of dose groups were selected to establish a concentration-effect relationship. It was not clear whether the methods were sufficiently sensitive for biosimilarity assessment in terms of detecting any potential differences. As per 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), it is important that assays used for comparability testing are demonstrated to have appropriate sensitivity to detect any

relevant differences and that experiments are based on a sufficient number of dilutions per curve to characterise the whole concentration-response relationship accurately. The applicant was asked to demonstrate that the submitted methods performed for biosimilarity assessment of glucose uptake, lipogenesis, phosphorylation of signaling intermediates were appropriately qualified for the purpose of comparability. The applicant justified the selected number of tested concentrations and dose range in the glucose uptake, lipogenesis and signalling assays by referring to previous bibliographical references where the methods were technically validated using these doses. Additional submitted literature together with newly submitted studies of methods development are considered sufficiently supportive for pivotal comparative studies. The data showed similarity between Insulin Human Rechon batches and the reference Humulin® Regular product batches.

Inhibition of lipolysis: Raw data as well as values of mean ± standard deviation of nM concentration of glycerol in the culture medium were represented. Obtained mean values for glycerol release lie within mean values of reference Humulin® Regular batches. However, it was not clear why solely the results at 1 nM insulin concentration were selected and shown in biosimilarity assessment, since results for other concentration may provide different output. It was also not clear whether the method is sufficiently sensitive for biosimilarity assessment in terms of detecting any potential differences. As per 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), it is important that assays used for comparability testing are demonstrated to have appropriate sensitivity to detect any relevant differences and that experiments are based on a sufficient number of dilutions per curve to characterise the whole concentration-response relationship accurately. Further details on how the method was qualified for intended purpose were asked to be provided. The applicant justified the selected single dose in this assay by referring to a previous bibliographical reference where the method was technically validated using this dose. Considering different cell type model described in literature, the selection of single concentration in comparability study between the test and reference products has not been sufficiently justified. However, based on comprehensive comparability exercise, this experiment per se does not preclude the claim of biosimilarity between tested Insulin Human Rechon and the reference Humulin® Regular products.

Glucose uptake, lipogenesis, signalling assays: In the main study 2021-10-28, the relative potencies of the compounds were calculated toward an internal control. Mean relative values with SD were presented without actual data of mean values and 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 control makes the comparison between Insulin Human Rechon and Humulin® Regular indirect. The applicant was asked to provide the means of actual data with their standard errors values of Insulin Human Rechon and the reference compounds to allow an adequate comparison between Humulin® Regular and Insulin Human Rechon samples for glucose uptake, lipogenesis, signaling assays. The means of actual data with their standard errors (n=3) values for glucose uptake, lipogenesis, signalling assays in the Study 2021-10-28 were demonstrated. The data showed to be similar between Insulin Human Rechon batches and the reference Humulin® Regular batches.

Finally, in Study 2021-10-28-Rechon the applicant calculated standard deviations for all experimental results. However, the experimental results were normalised to controls that were included in each separate experimental run. The applicant did not discuss how the standard deviations were calculated for these data. It is understood that the tested insulin (Lilly and Rechon insulin) and the controls were run in triplicates (n=3 replicates per treatment). Both the means of the experimental results and the control therefore themselves have standard deviations. When normalising the triplicate means of the tested insulin products with the control triplicate mean, the standard deviations of both means would

therefore influence the overall standard deviation of the normalised mean value. It cannot be excluded that the applicant solely divided the insulin mean values and their standard deviations with the mean of the controls and disregarded the standard deviation of the control mean. This would be incorrect and would underestimate the standard deviation of the normalised mean values.

The applicant's response to this concern was not satisfactory (it is still not apparent how standard deviations of normalised results were calculated in this experiment). Even if the standard deviations of the insulin (Lilly and Rechon insulin) samples in the different experiments of Study 2021-10-28-Rechon were higher than reported, in vitro biosimilarity between the Lilly and Rechon insulin products would presumably have been demonstrated in this study. Nonetheless, for the sake of correctness, the applicant was again expected to provide documentation on how the standard deviations of all controlled values in Study 2021-10-28-Rechon were calculated. This request was again not satisfactorily answered and no documentation of the applicant's calculation was provided. As this aspect will not influence the outcome of this procedure, it was ultimately not further pursued even though no meaningful response was received by the applicant. As a consequence, all samples that were normalised to control (and are expressed as % response relative to control) could bear an incorrect calculation of their standard deviation.

Pharmacokinetics

The applicant did not submit studies on the pharmacokinetics of Insulin Human Rechon which is in compliance with the EMA "Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues" (EMEA/CHMP/BMWP/42832/2005 Rev1).

Toxicology

Although according to the guideline (EMEA/CHMP/BMWP/32775/2005_Rev. 1), no toxicity studies were needed in the case of Insulin Human Rechon, the applicant provided two repeat dose studies in rat, which were performed in 2012, before the guideline was published.

In the scientific advice procedure in 2010, it was recommended that if three dose levels were included in the main study, a pre-study would not be needed. As it was indicated that Maximum Tolerable Dose (MTD) could vary between laboratories, a range finding study could be used to find the MTD. However, the provided 1-week dose-range finding study was performed only with one dose, so it could not be used to define the MTD.

Overall, all test articles involved in the 4-week repeat-dose toxicity study in rats, Insulin Human Rechon and another formulation, and the innovator products Humulin® Regular were well tolerated at doses of 1.2 and 2.5 IU/kg b.w./day and no test-article related adverse events were observed, however, *in vivo* data may not be sensitive enough for any conclusions on biosimilarity. Also, toxicokinetic evaluation revealed largely similar results for all test-articles. However, the analysis of the results was hampered by very small experimental groups (2-3 animals per time point analysed) and the outcome has therefore be regarded with caution. Of note, only two dose levels were used in the pivotal study. As suggested in the scientific advice, preferably three dose levels for both test and reference product are recommended to reduce risk of failure due to variability between dose groups. For repeated dose toxicity studies where less than three dose levels are evaluated, the high dose should be selected at the high end of the dosing range. But no toxicity was observed in any of the test groups in the pivotal study and the high dose of 2.4 IU/kg/day was defined as the NOAEL. Therefore, since the MTD in the context of the pivotal study is unknown, no toxicology conclusions can be based on this study. Considerable variability was observed regarding animal exposure data between individuals but also between male and female groups. Pharmacokinetic data units from the repeat-dose toxicity study have been transformed to be comparable to human data, but the animal-to-human

exposure comparison and exposure margin calculations were not provided by the applicant. Nevertheless, by the Assessor's calculation, the exposure margins are quite low in the terms of AUC (4x), but they are satisfactory in terms of C_{max} (22x). Thus, it can be concluded that the exposure margin in the repeat dose toxicity study as compared to clinical data (Study RCT-001) is sufficiently high based on C_{max} values. Therefore, toxicity data from the 4-week repeat-dose toxicity study in rats can be used as supporting information, indicating, that no unforeseen toxicity of Insulin Human Rechon is to be expected.

The omission of studies on genotoxicity, carcinogenicity and reproductive toxicity is accepted and in line with EMA "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 applicant confirms that impurities are controlled within the specification limits, however, a justification for the proposed acceptance criteria is missing (please refer to the Quality LoQ).

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, Insulin Human Rechon is not expected to pose a risk to the environment.

3.2.7. Conclusion on non-clinical aspects

The submitted non-clinical data support the conclusion on biosimilarity between the proposed biosimilar product and the reference insulin product. However, the experimental results in the in vitro biological activity assay that were normalised to controls may have incorrect calculation of their standard deviation.

3.3. Clinical aspects

The applicant developed a fast-acting human insulin (Insulin Human Rechon, hereafter referred to as Insulin Human Rechon) as biosimilar to the European reference product Humulin® Regular (Eli Lilly and Company Ltd) for approval in the European Union (EU). The reference product Humulin® Regular was approved for market authorization in Sweden on 31st March 1987 and the Swedish Humulin® Regular is used as reference product. This biosimilar marketing authorisation application (MAA) is submitted via Centralised Procedure in accordance with Article 3(1) of Regulation (EC) No 726/2004 and under Article 10(4) of Directive 2001/83/EC. Insulin Human Rechon is a new insulin product, not previously marketed in Europe. Insulin Human Rechon contains the active substance soluble human insulin produced by recombinant DNA technology in transformed Escherichia coli bacteria. It is formulated as 100 IU/ml solution for injection in a 3 ml cartridge equivalent to 300 units of soluble insulin. Per SmPC, the presented formulation in cartridges is only suitable for subcutaneous injections from a reusable pen. The dosage should be determined by the physician, according to the requirement of the patient. The applicant applies for the following indication: *for the treatment of patients with diabetes mellitus who require insulin for the maintenance of glucose homeostasis.* The proposed indication is identical to the approved reference product Humulin ® Regular as stated in the respective SmPC.

The applicant presented the following clinical development programme:

- One combined pharmacokinetic (PK) and pharmacodynamic (PD) euglycaemic clamp biosimilarity study in healthy volunteers (Study Code RCT-001) with the primary objective to demonstrate similar PK and PD between Insulin Human Rechon and Humulin® Regular.
- One 7-month safety and immunogenicity study in patients with T1DM (Study Code RCT-004) with the primary objective to assess the long-term safety of Insulin Human Rechon as compared to Humulin® Regular in terms of immunogenicity and insulin tolerance.

A summary of the clinical program is provided in the table below. Details of the clinical trial design and results will be presented in the following sections.

- **Tabular overview of clinical studies**

Table 2: Overview Listing of Clinical Studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regiment; Route of Administration	Number of subjects	Healthy subjects or Diagnosis of patients	Duration of treatment	Study Status; Type of Report
PK/PD	RCT-001 (addendum 1 and 2 of RCT-001)	5.3.4.1 eCTD	Demonstrate similar PK and PD between Rechon Insulin Human Soluble and Humulin® Regular. Assess safety and tolerability of Rechon Insulin Human Soluble in single doses.	The pilot study: single dose. The main study: randomised, double-blinded, cross-over, active-controlled.	Rechon Insulin Human soluble, solution for injection, 0.3IU/kg, single dose, subcutaneous	Pilot study: 3 Main study: 22	Healthy subjects	Single dose, two period.	Complete; Final CSR.
Safety	RCT-004 (addendum RCT-004)	5.3.5.1 eCTD	Asses long-term safety of Rechon Insulin Human Soluble compared to Humulin® Regular in terms of immunogenicity, insulin tolerance and general safety variables.	Randomised, open, parallel-group, active-controlled.	Rechon Insulin Human Soluble, solution for injection, in 3mL cartridges with individualised dosing based on glucose levels, subcutaneous.	304	Patients with type 1 Diabetes Mellitus	7 months approximate (5 visits)	Complete; Final CSR.

3.3.1. Clinical pharmacology

One combined PK/PD clinical trial (study code RCT-001) was conducted to demonstrate PK and PD equivalence of the biosimilar (Insulin Human Rechon) and reference medicinal product (Humulin® Regular). The RCT-001 study program consisted of a pilot study, followed by the main study: a double-blind cross-over design in normal healthy volunteers using a single subcutaneous dose (0.3 IU/kg) as main study. PD biosimilarity was assessed using a euglycaemic clamp technique. The pilot study served to optimise the euglycaemic clamp protocol. Safety and tolerability were additionally compared between Insulin Human Rechon and Humulin® Regular.

The clinical trial was conducted in accordance with GCP and the requirements of Directive 2001/83/EC Annex I, as amended by Directive 2003/63/EC and Directive 2001/20/EC, and all applicable regulations.

3.3.1.1. Analytical methods

Study RCT-001

Serum Insulin and C-Peptide

Concentrations of insulin and C-peptide in plasma samples were measured using an immunometric sandwich method

Plasma Glucose

Plasma glucose concentrations were determined using a kit.

3.3.1.2. Pharmacokinetics

Absorption

Bioavailability

Bioavailability was assessed in the scope of the comparative bioequivalence study RCT-001 evaluating the PK and PD biosimilarity of Insulin Human Rechon (Test) versus Humulin® Regular (Reference). For more details refer to the following section regarding *Bioequivalence*.

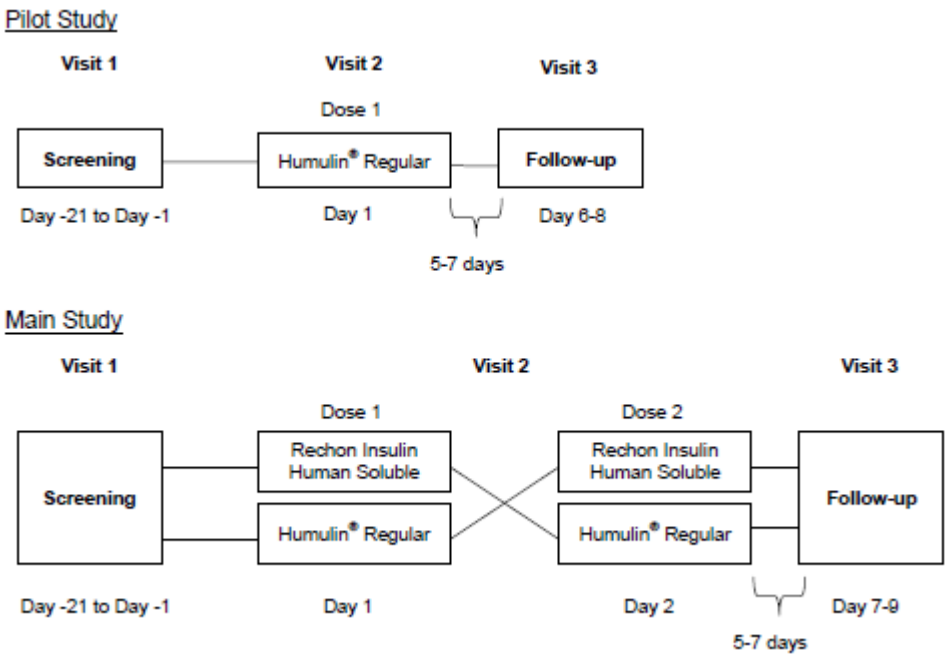
Bioequivalence

Study RCT-001 was a single-center Phase 1, bioequivalence trial evaluating the PK and PD of Insulin Human Rechon (Test) versus Humulin® Regular (Reference) in healthy adult subjects. The safety and tolerability profiles of the proposed biosimilar Insulin Human Rechon were also compared to the ones of Humulin® Regular.

Study design

The study consisted of a pilot study, followed by a main study. The overall study design of the pilot and main study is displayed in the Figure below.

Figure 6: Overall study design



Pilot Study

The purpose of the pilot study was to optimise the protocol for the euglycaemic clamp technique and to determine the procedure for adjustment of glucose infusion rate (GIR). The pilot study was a single centre, single dose, euglycaemic clamp study planned with 4 subjects. The subjects received one subcutaneous dose of Humulin® Regular as 0.3 IU/kg body weight. The pilot study was performed for methodology reasons only and there was no intention to collect the case report forms (CRFs) from the pilot study and to present data in the clinical study report.

Main Study

The main study was a single centre, randomised, double-blind, single dose, two-period, crossover, euglycaemic clamp study planned for approximately 22 healthy subjects, investigating the PK and PD similarity between the two fast-acting insulins Insulin Human Rechon and Humulin® Regular. The study included 3 visits: a screening visit to assess the eligibility of the subjects (Visit 1), an overnight treatment visit (Visit 2) and a follow-up visit with final examinations (Visit 3). Subjects had to fast overnight (22:00 hours until IMP administration). During the clamp procedure, the first dose (0.3 IU/kg) of either Insulin Human Rechon or Humulin® Regular was administered subcutaneously and the euglycaemic clamp then continued for 10 h with a target plasma glucose of 6 mmol/L. The subjects stayed overnight at the study site and the euglycaemic clamp study was repeated on the next day. The subjects received their second dose of the IMP (i.e. the IMP not given on Day 1) 24 h after the first dose. The washout period was therefore 24 hours. The overall study duration for each subject was 8-30 days including 3 visits and 1 overnight stay.

Study population

The study population included healthy males or females, between 18 and 60 years of age, with a body mass index of 18.0-28.0 kg/m2 and a weight of 50-100 kg, non-users of nicotine products and without any clinically significant disease. Subjects were selected according to the inclusion and exclusion criteria as pre-specified in the protocol after having given written informed consent. For the main study, a sufficient number of subjects was to be enrolled to ensure evaluable data for approximately 22 subjects.

Study drugs and treatment

Test Product

100 IU/ml Insulin Human Rechon solution, packed into vials

Reference Product

100 IU/ml Humulin® Regular solution (Market authorization holder: Eli Lilly and Company;, packed into vials. Reference product holds a market authorization in Sweden.

Table 3: Investigational Medicinal Products

	Investigational Medicinal Product	
Product name	Insulin Human Rechon	Humulin® Regular
Source	*E.Coli*	*E.Coli*
Dosage form	Injection solution	Injection solution
Unit strength	100 IU/ml	100 IU/ml
Appearance	Clear colorless solution	Clear colorless solution
Excipients	Glycerol M-cresol Hydrochloric acid Sodium hydroxide Water for injection	Glycerol M-cresol Hydrochloric acid Sodium hydroxide Water for injection
Administration	Subcutaneous injection	Subcutaneous injection
Mode of action	Fast-acting	Fast-acting
Individualised dosage	Subjects received their injections of IMPs in a double-blind manner, in accordance with the randomization list. The total dose was dependent on the body weight of the subject and was 0.3 IU/kg body weight.	

Dose and treatment

Each subject was to receive a single, subcutaneously injected, dose of 0.3 IU/ kg body weight of either study drug on two separate treatment periods. Dosing of the individual subjects was standardised and took place at the same time point (approximately 10am) for all subjects. Subjects were fasting overnight (starting at 10pm) before the dosing and during the euglycaemic clamp study. The subjects received a standardised meal when the euglycaemic clamp study was over. Only water consumption was allowed until the end of the clamp. The IMP was administered as subcutaneous injection into in the middle abdominal area (the first dose was to be injected in the left side and the second dose in the right side) using a syringe.

For glucose infusion and plasma glucose measurements during euglycaemic clamp, two catheters were inserted intravenously; one in the dorsal hand vein for blood sampling and the other in an antecubital vein of the contralateral arm for infusion of glucose (200 mg/ml). The plasma glucose level was maintained at the target level of 6 mmol/L. For details regarding the euglycaemic clamp please refer to the dedicated section below.

Blood sampling and other Study Procedures

Study procedures

The planned study flow chart for the main study is presented in the table below.

Table 4: Study Flow Chart of the Main Study

Procedure		Visit 1 Screening	Visit 2								Visit 3 Follow-up
		Day -21-Day -1	Day 1, Dose 1				Day 2, Dose 2				Day 7-9
			Before dose	0 h	0-10 h	After clamp	Before dose	0 h	0-10 h	After clamp	
Informed Consent		X									
Inclusion/ Exclusion		X	X[a]								
Demographics		X									
Weight		X	X								
Height, BMI		X									
Medical History		X	X[b]								
HIV Screen Hepatitis B and C		X									
Urine Drug Screen		X	X								
Alcohol Breath Test		X	X								
Pregnancy Test		X	X								X
Physical Examination[c]		X									X
Vital Signs[d]		X									X
ECG		X									X
Laboratory Safety Assessments[e]		X	X[f]		X[g]		X[f]		X[g]		X
Randomisation			X								
IMP Injection[h]	Glucose Clamp			X				X			
PK Blood Sampling for Insulin and C-peptide[i]	Glucose Clamp		X		X		X		X		
Blood Sampling for Plasma Glucose[i]	Glucose Clamp		X	X	X		X	X	X		
Glucose Infusion	Glucose Clamp		X	X	X		X	X	X		
Overnight Fasting			X				X				
Standardised Meal						X				X	
Discharge										X[k]	
Concomitant Medication		X[l]	←							→	
AE Assessment[m]			←							→	

AE = Adverse Event, BMI = Body Mass Index, ECG = Electrocardiogram, HIV = Human Immunodeficiency Virus, IMP = Investigational Medicinal Product, PK = Pharmacokinetics

[a] Recheck of eligibility criteria

[b] Change from previous visit (pre-treatment events)

[c] Includes assessment of the general condition, ears, nose, throat, heart, lungs and abdomen

[d] Includes systolic and diastolic blood pressure and oral temperature. The subjects should lay down for 5 min before the measurements

[e] All laboratory safety measurements are listed in Table 6

[f] Blood sampling for haemoglobin, CRP and potassium only

[g] Blood sampling for potassium only. Will be performed at 2 h, 4 h and 8 h post dose.

[h] Subcutaneous injection of either Rechon Insulin Human Soluble or Humulin® Regular

[i] Blood samples will be drawn at the following time points: -15 min, 0 h (pre-dose) and 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 120 min, 150 min, 3 h , 4 h, 5 h, 6 h, 7 h, 8 h and 10 h post-dose

[j] Glucose blood samples will be drawn approximately every 5 minutes from hours -2 to 4 and every 10 minutes from hours 4-10. Additional samples may be drawn if deemed necessary and fewer samples may be drawn if considered sufficient for the maintenance of the glucose level at the target concentration (see Section 10.2.2)

[k] Subjects may leave the study site 12 h after the dose.

[l] Including prior medication to confirm eligibility according to the inclusion/exclusion criteria

[m] AEs (including evaluation of local intolerance) will be recorded from the first dose of IMP and until the follow-up visit

Blood sampling

- Insulin and C-peptide

The date and actual time point for each sampling were to be recorded in the CRF. At each sampling, approximately 3.5 mL of venous blood was collected from the subjects into SSD tubes with gel. After 30-45 minutes, each blood sample was centrifuged at 2000 g for 10 minutes. Separated plasma was transferred into tubes and stored at -20°C or below. Insulin and C-peptide levels were determined from the same sample. The blood sampling schedule was planned as follows:

Table 5: Scheduled PK Blood Sampling for Insulin and C-peptide

Time (h)	Time (min)	Approximate sampling hour	IMP Injection	Blood sampling, insulin and C-peptide
	-15			X
0	0	10:00	X	X (pre-dose)
	15			X
	30			X
	45			X
1	60	11:00		X
	75			X
	90			X
2	120	12:00		X
	150			X
3	180	13:00		X
4	240	14:00		X
5	300	15:00		X
6	360	16:00		X
7	420	17:00		X
8	480	18:00		X
10	600	20:00		X

- Glucose

Blood sampling for plasma glucose determination was via an intravenous catheter during the euglycaemic clamp procedure. The study subject was in the supine position and the hand for glucose sampling was kept warm with a heating pad to arterialize venous blood. Blood sampling for plasma glucose determination was approximately every 5 minutes from hours -2 to 4 and every 10 minutes from hours 4-10. Deviations from the target plasma glucose level (10 %) during the clamp study resulted in more frequent blood samplings than scheduled. Also decreased sampling frequency was allowed, if considered appropriate.

Euglycaemic Glucose Clamp

The PD of Insulin Human Rechon and Humulin® Regular was measured using a manual euglycaemic clamp technique. The procedure was performed in the supine position. Two catheters were inserted intravenously; one in the dorsal hand vein for blood sampling (this hand was kept warm with a heating pad to arterialise venous blood) and the other in an antecubital vein of the contralateral arm for infusion of glucose (200 mg/mL). (The procedure was slightly modified in protocol amendment 2.)

Analyses of blood glucose concentrations were performed continuously during the clamp study. An intravenous infusion of glucose (200 mg/mL) was adjusted to maintain the subject's plasma glucose at a target level of 6 mmol/L and the GIR was recorded. After instrumentation, glucose infusion started and after 2 h the IMP was administered subcutaneously. Plasma glucose concentrations were determined approximately every 5 min from hours -2 to 4 and every 10 min from hours 4 to 10 .

The plasma glucose level was maintained at the target level of 6 mmol/L. The target plasma glucose level was kept constant by varying the infusion rate of glucose. The infusion rate was adjusted after plasma glucose determinations according to the algorithm in the table below. The algorithm for the clamp study was developed during the pilot study and further optimised during the main study to fit

the actual clinical treatment of the subjects. It should be noted that some subjects needed infusion rate changes not covered by the ranges in the algorithm.

Table 6: Algorithm for Glucose Infusion Rate Adjustments

Time Interval	Glucose Level (mmol/L)	Adjustments
-120 min – 0 min[a] GIR start: 2 mg/kg/min	6.8-7.2	Decrease GIR with 20%
	6.3-6.7	Decrease GIR with 10%
	5.8-6.2	No action
	5.3-5.7	Increase GIR with 10%
	4.8-5.2	Increase GIR with 20%
0-2 h after IMP administration[b]	7.3-7.8	Decrease GIR with 30%
	6.8-7.2	Decrease GIR with 20%
	6.3-6.7	Decrease GIR with 10%
	5.8-6.2	No action
	5.3-5.7	Increase GIR with 10%
	4.8-5.2	Increase GIR with 20%
	Below 4.8	Increase GIR with 50%
2-4 h after IMP administration[c, d]	6.8-7.2	Decrease GIR with 20%
	6.3-6.7	Decrease GIR with 10%
	5.8-6.2	No action
	5.3-5.7	Increase GIR with 10%
	4.8-5.2	Increase GIR with 20%
4-10 h after IMP administration[d]	6.8-7.2	Decrease GIR with 20%
	6.3-6.7	Decrease GIR with 10%
	5.8-6.2	No action
	5.3-5.7	Increase GIR with 10%
	4.8-5.2	Increase GIR with 20%

a Do not change infusion rate until 5 min after start

b Increase infusion rate with 100% 10 min after injection. Consider increase infusion rate with 100% 40 min after injection

c Two blood glucose values outside the range "no action" needed for a change

d If change is >1 from last measurement, take a new blood sample or make new analysis

Study objectives and endpoints

Objectives

The primary objective was to demonstrate similar PK and PD between Insulin Human Rechon and Humulin® Regular, when given as single subcutaneous doses to healthy subjects.

The secondary objective was to assess the safety and tolerability of single subcutaneous doses of Insulin Human Rechon.

PK and PD variables assessment was only intended for subjects in the main study.

PK endpoints

Primary PK Variable

AUC from time zero to 10 h (AUC0-10 h) for Insulin Human Rechon as compared to Humulin® Regular.

Secondary PK Variables

Cmax, time to Cmax (tmax), terminal half-life (t1/2), AUC(0-2 h), AUC(2-4 h), AUC(0-4 h), AUC(4-10 h), AUC(0-∞) of Insulin Human Rechon as compared to Humulin® Regular.

PD endpoints

Primary PD Variable

AUC for GIR from time zero to 10 h (AUC-GIR(0-10 h)), for Insulin Human Rechon as compared to Humulin® Regular.

Secondary PD Variables

Maximum GIR (GIRmax), time to GIRmax (GIR-tmax), AUC-GIR(0-2 h), AUC-GIR(2-4 h), AUC-GIR(0-4 h) and AUC-GIR(4-10 h) for Insulin Human Rechon as compared to Humulin® Regular.

Sample size

The comparison of Insulin Human Rechon (T) and the reference product Humulin® Regular (R) with respect to PK/PD variables (AUC and AUC_{GIR} 0-10 h) was performed using a 2x2 cross-over design. Both Insulin products were considered equivalent if the 90% CI for the ratio AUC_T /AUC_R for the PK variable AUC(0-10 h) fell in the interval 0.80-1.25. On the natural logarithm (ln) scale, the corresponding 90% CI was -0.223-0.223. Based on previous studies, it was assumed that the intra-individual CV for AUC(0-10 h) was about 0.18. CV was transformed into mean square error (MSE) by the equation MSE = ln(1 + CV2). To achieve a power ≥90%, 9 subjects were required per sequence group to determine the precision provided the true ratio was within a range of 95-105%. An intra-individual CV of the ln-transformed AUC_{GIR}(0-10 h) was estimated to 20%. Subsequently 10 subjects were required in each sequence group in the study to determine the precision in such way that 90% CI of the ratio fell within an acceptance range of 80-125 % with a power of ≥90% provided the true ratio was within a range of 95-105%.

To compensate for possible drop-outs, one subject was added for each treatment sequence group. The analysis population for each period comprised 11 subjects. In total 22 subjects were to be included in the study. The calculation was based on the TOST procedure in nQuery Advisor version 5.0.

Randomisation and blinding

At the screening visit, screening numbers (Sxx) were assigned sequentially to the subjects.
Before dosing, eligible subjects were assigned a subject number determining the treatment according to the randomisation list. Subject numbers were assigned sequentially to the subjects as they enter the study. Subjects were randomised according to a computer-generated randomisation to the treatment sequence such that half of the subjects would start their treatment with Insulin Human Rechon and half of the subjects will start their treatment with Humulin® Regular.

The study was double-blind such as subjects, clinical site staff, the Investigator and the sponsor were blinded regarding the study medication each subject receives. However, an assigned person without any other involvement in the study prepared the doses and handed them over to the person responsible for administration.

Procedures for unblinding in the event of an emergency were foreseen in the study protocol.

Prior to code breaking, a blind review of the data was performed. The objective of the review was to identify problems and to make decisions regarding data analytical issues under blind conditions. The blind review included the following topics:

- Exclusion of subjects from the FAS and PPS was discussed
- Reported protocol deviations were reviewed to determine if any of them warranted exclusions from the analysis sets
- It was decided that results from the haemolysed blood samples for insulin determinations

should be deleted from the analysis database and not used for the calculation of PK variables
- The baseline period for GIR was set to the last 60 min before study medication administration
- The parameters for the LOESS smoothing of GIR curves were chosen: local polynomial of degree=2 and smoothing factor=0.2. Furthermore, baseline was given 10 times the weight of other data points

All issues and decisions taken during the blind review were documented in the Pre-Analysis Review document

Pharmacokinetic and pharmacodynamic data analysis

Calculation of the primary PK / PD parameters

Plasma insulin concentration-time data were analysed by non-compartmental methods with linear interpolation and uniform weighting using model 200 (extravascular administration) of WinNonlin (Pharsight Corporation, California, United States of America).

As insulin is an endogenous substance, the PK parameters were determined using baseline-corrected concentrations. In accordance with the standard subtractive baseline correction method, the mean of the individual pre-dose concentrations were subtracted from each post-dose assessment. Following baseline correction, negative serum concentrations occurring before C_{max} were treated as zero and negative serum concentrations occurring after C_{max} were omitted from the analysis.

C_{max} and t_{max} were derived from the observed serum concentration data. $AUC_{(0\text{-}10\ h)}$, $AUC_{(0\text{-}2\ h)}$, $AUC_{(2\text{-}4\ h)}$, $AUC_{(0\text{-}4\ h)}$ and $AUC_{(4\text{-}10\ h)}$, were calculated using linear trapezoidal interpolation.

$AUC_{(0\text{-}\infty)}$ was calculated using linear trapezoidal interpolation to the last point showing a measurable plasma concentration and then extrapolated to infinity according to the following formula:

$AUC_{(0\text{-}\infty)} = AUC_{0\text{-}t} + AUC_{extra}$, where $AUC_{extra} = C_t/\lambda_z$.

For $AUC_{(0\text{-}10\ h)}$ the areas were calculated to the last point showing a measurable plasma concentration and when necessary extrapolated to 10 h, respectively the estimated concentration in the last quantifiable sample and λ_z. If the end time of the area interval occurs within the range of the data but did not coincide with an observed data point, then a linear interpolation was performed to estimate the corresponding concentration.

λz is the first order rate constant associated with the terminal portion of the curve and was estimated via linear regression of time versus log concentration.

Prior to calculation of PD parameters the profiles were smoothed using the LOESS smoothing technique with a smoothing parameter to be fixed during blind review. A local polynomial of degree=2 and smoothing factor=0.2 were used, and baseline was given 10 times the weight of other data points.

The same specifications apply to GIR as to the PK data, with the exception of the estimation of the terminal portion of the curve. For $AUC\text{-}GIR_{(0\text{-}10\ h)}$ and $AUC\text{-}GIR_{(4\text{-}10\ h)}$, the areas were calculated to the last point showing a positive baseline-corrected value and when necessary extrapolated to 10 h, using the estimated value at the last point with a positive baseline-corrected value and an estimated elimination rate (similar to λ_z for PK data).

Statistical methods

Data Sets to be analysed

The full analysis set (FAS): All randomised subjects who have received at least one dose of study medication and have at least one post-baseline assessment of the primary PK and PD variables.

Per protocol set (PPS): All subjects who fulfilled all inclusion criteria but none of the exclusion criteria, complied with study medication dosing scheme and completed the study without major protocol violations. Subsequently, subjects who did not provide evaluable data for both the test product and the reference product were not included in the PPS.

Deviations to the study protocol were documented in a Protocol Deviation Log. The classification of subjects into protocol violators were made during a meeting before database lock upon prior breaking the randomisation codes. The classification will be mutually agreed between the Sponsor and TFS before breaking the randomisation codes.

The final decision regarding which protocol deviations that warranted exclusions from the PPS was taken during the pre-analysis review. During this review, the analysis populations were finalised and any changes to the analysis plan documented.

Safety set: all randomised subjects who receive at least 1 dose of study medication and for whom any post-dose safety data were available. Safety summaries were performed on the safety set.

The primary objective was analysed using the PPS and all conclusions regarding biosimilarity were based on the results from the analyses on the PPS.

Primary efficacy analysis

Biosimilarity was tested by applying Schuirmann's two one-sided test (TOST) procedure[1].

To test bioequivalence between Insulin Human Rechon and Humulin® Regular, an ANOVA for a 2X2 crossover design was conducted for the ln-transformed $AUC_{(0\text{-}10\ h)}$ and ln-transformed $AUC\text{-}GIR_{(0\text{-}10\ h)}$.

The probability of exceeding the limits of acceptance (80-125%) was obtained using the TOST method. The products were considered bioequivalent with both p-values ≤ 0.0500.

The 90% confidence interval (CI) in the bioequivalence range had to be the same as the rejection of both null hypotheses at α (=0.05) level by two one-sided tests (TOST). This method means that two tests were performed:

1) H_{0A}: The geometric mean ratio of AUC lies above 1.25
2) H_{0B}: The geometric mean ratio lies below 0.8.

If both these hypotheses were rejected, then the conclusion was that the ratio lies in the interval [0.8, 1.25].

The two treatment periods were separated by a wash-out period of 24 h which was sufficient to ensure drug concentrations below the limit of detection. Thus a test for carry-over effects was not considered relevant. The potential carry-over has been directly addressed by examination of the pre-

[1] Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm. 1987;15(6):657-80

treatment plasma concentrations for the second dose. If there was a carry-over effect only the first period data provided unbiased information.
The significance level for the statistical testing of the primary objective was 0.0500. In the secondary PK/PD analyses, no adjustment for multiplicity was considered. The basic distributional assumption underpinning the analysis was that the logarithm of AUC follows a normal distribution.
The primary variables were presented as geometric means and confidence intervals for the ratio of the two treatment means. Furthermore, graphs displaying individual profiles were produced.

All secondary analyses were performed to support the primary analyses. The PK and PD parameters were analysed in the same manner as the primary PK variable. However, the confidence intervals were not related to pre-defined limits, with the exception of C_{max} and GIR_{max}. C_{max} and GIR_{max} were ln-transformed and an ANOVA was applied. TOST and 90% CI for the ratio of product averages of C_{max} and GIR_{max} were calculated.

Protocol amendments

Final protocol version 1 was issued on 11. October 2012. The protocol was amended twice with substantial amendment 1 issued on 29. January 2013 and non-substantial amendment 2 on 10. April 2013. The final protocol and the subsequent protocol versions were presented.

Substantial Amendment 1

The amendment described changes of the Principal Investigator and clinical trial site, including laboratories for safety and PK analyses. Changes to the blood sample and euglycaemic clamp procedures (volume of blood sampling; switch from 20 % dextrose to 200 mg/ml glucose) were also made. Text alterations and added information were provided.

Non-substantial Amendment 2

The protocol amendment concerned minor corrections in the protocol for administrative purposes.

Study results

Addendums to clinical study report

Addendum 1

The PD analyses described in the final study report were repeated with a 95% CI for the ratio between the two treatments. See also section 2.2.3.

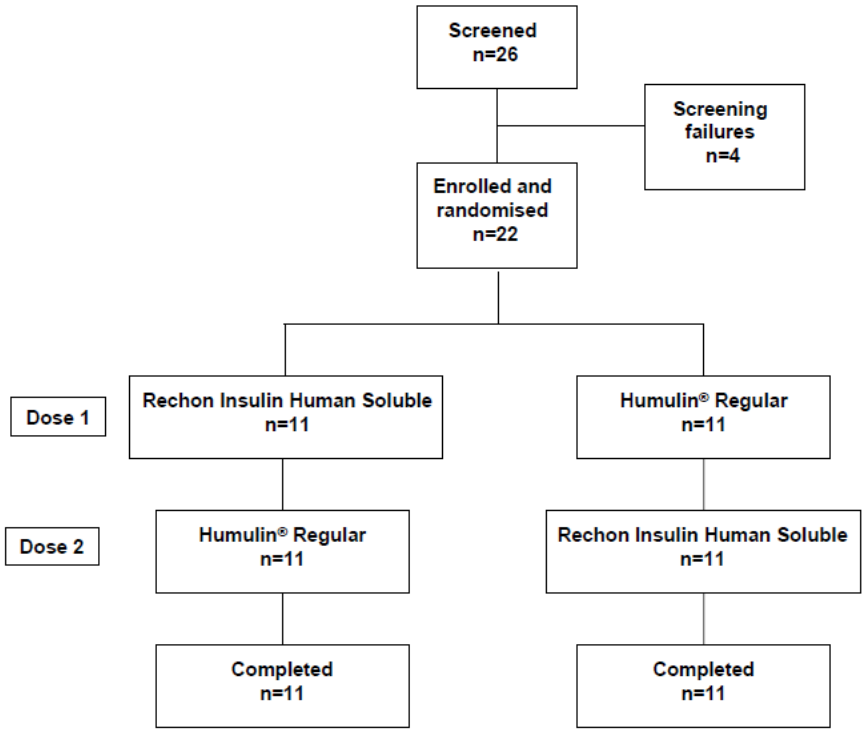
Addendum 2

Issued and written because the name of the manufacturing company of the active substance (insulin human) of Rechon Insulin was incorrect in the final clinical study report.

Disposition of subjects

A total of 26 subjects were screened and 22 subjects were included in the main study of which 11 subjects were in each treatment sequence. All 22 subjects performed all visits in the study and completed the study according to the protocol. All 22 subjects were included in the FAS, PPS and safety sets and no subjects were excluded from PK and PD analysis sets.

Figure 7: Subject disposition



Source: Table 14.1.2 and Screening and Enrolment Log

Baseline characteristics

Baseline characteristics included age, sex race, weight (kg), height (cm) and BMI (kg/cm^2). Descriptive statistics were presented for continuous variables; frequency counts and percentages for categorical values (sex and race). An overview of the demographic data is displayed in the table below.

Table 7: Demographic data (safety set)

		Rechon Insulin Human Soluble/Humulin® Regular (n=11)	Humulin Regular/Rechon Insulin Human® Soluble (n=11)	Total (n=22)
Age	No. of obs.	11	11	22
	Mean (sd)	26.4 (5.5)	27.2 (8.9)	26.8 (7.2)
	Median	26.0	25.0	25.5
	Q1, Q3	23.0, 27.0	21.0, 29.0	21.0, 28.0
	Min, Max	20, 37	20, 48	20, 48
Sex	No. of obs.	11	11	22
	Female	3 (27.3%)	4 (36.4%)	7 (31.8%)
	Male	8 (72.7%)	7 (63.6%)	15 (68.2%)
Race	No. of obs.	11	11	22
	Black or African American		1 (9.1%)	1 (4.5%)
	White	11 (100.0%)	10 (90.9%)	21 (95.5%)
Weight (kg)	No. of obs.	11	11	22
	Mean (sd)	74.8 (8.1)	75.8 (11.2)	75.3 (9.5)
	Median	74.7	76.2	75.3
	Q1, Q3	69.9, 80.2	67.0, 85.7	69.9, 80.3
	Min, Max	61, 91	53, 90	53, 91
Height (cm)	No. of obs.	11	11	22
	Mean (sd)	176.4 (8.4)	178.6 (9.1)	177.5 (8.6)
	Median	180.0	180.0	180.0
	Q1, Q3	170.0, 182.0	171.0, 184.0	171.0, 183.0
	Min, Max	162, 188	163, 196	162, 196
Body Mass Index (kg/m^2)	No. of obs.	11	11	22
	Mean (sd)	24.0 (2.2)	23.7 (2.6)	23.9 (2.3)
	Median	24.2	23.5	23.9
	Q1, Q3	22.5, 25.8	22.5, 26.5	22.5, 25.8
	Min, Max	20, 27	20, 28	20, 28

Source: Table 14.1.3

Deviations

A few minor protocol deviations (PDs) were reported, which were documented in the Declaration of Database Lock document.

There were 15 PDs in total in 12 subjects. Of the PDs, the following 9 PDs relate to the primary study assessments:

a. some glucose infusion received subcutaneously for dose 1 and at dose (2 PDs).

b. a PK sample not taken (5 PDs),

c. glucose leakage during GIR for , and the cannula was changed once discovered

d. day 2 pre-dose sample taken a few min after the dose for Also noteworthy, which was not mentioned as a protocol deviation:

- "It should be noted that for one subject, glucose infusion was changed to a too high rate at about 5 h after dosing on Day 1 (Rechon Insulin Human Soluble). When this was discovered, the infusion was temporarily stopped. The effect on GIR_{max} was minimised by the smoothing of the curves."

No deviations to eligibility criteria or other important deviations during the study were reported.

Measurements of Treatment Compliance

The subjects were dosed at the study site and received the IMPs under medical supervision. Injected amount of IMP and time and location of injection were provided per subject as list.

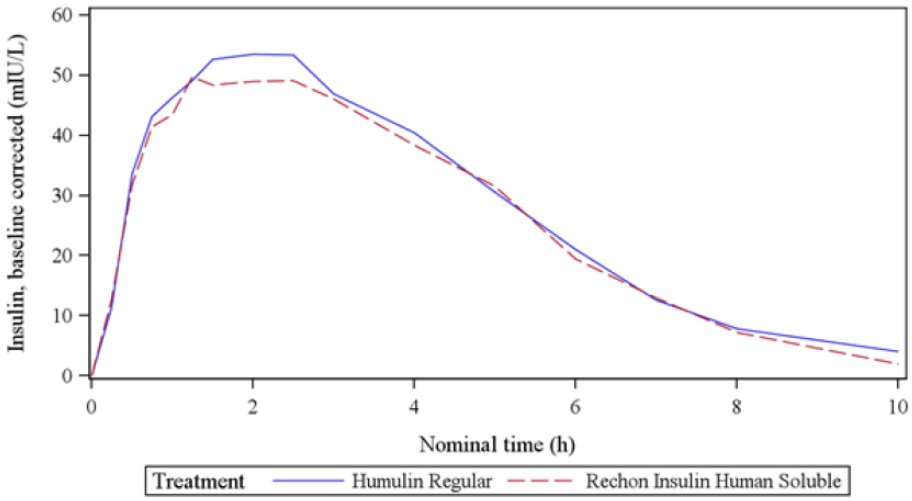
Pharmacokinetic results

All 22 subjects were included in the FAS, PPS and safety sets with no subject being excluded from PK analyses. However, 53 samples were removed from calculation of PK variables due to haemolysis.

Insulin serum concentrations

The plasma concentrations of Insulin Human Rechon and Humulin® Regular were measured during the euglycaemic clamp study after subcutaneous administration of the IMP (0.3 IU/kg body weight, single dose). The mean concentration-time profile was provided as comparative base-line corrected plot. The mean insulin plasma curve profiles were similar for Insulin Human Rechon and Humulin® Insulin as presented in the figure below.

Figure 8: Mean insulin plasma concentration versus time (PPS)



Source: Figure 14.4.7

Pharmacokinetic analysis results

Summary statistics for the primary PK variable AUC(0-10 h), the prioritized secondary PK variable Cmax and the other additional secondary PK variables were calculated for each variable and are presented in the table below. Mean t½ was longer for Humulin® Regular (1.8 h) than for Insulin Human Rechon (1.1 h), whereas median tmax was the same for the two IMPs (2.0 h).

Table 8: Summary statistics for PK variables

AUC(0-10 h) (hr*mIU/L)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	269 (44.9)	267 (35.5)
	Median	272	268
	Q1, Q3	231, 303	230, 298
	Min, Max	217, 362	221, 317
	CV(%)	16.7	13.3
	Geometric mean	266	265
2	No. of obs.	11	11
	Mean (sd)	255 (32.6)	278 (34.8)
	Median	249	292
	Q1, Q3	219, 284	238, 306
	Min, Max	217, 316	226, 322
	CV(%)	12.8	12.5
	Geometric mean	253	276
Total	No. of obs.	22	22
	Mean (sd)	262 (38.9)	273 (34.8)
	Median	253	271
	Q1, Q3	231, 286	238, 298
	Min, Max	217, 362	221, 322
	CV(%)	14.9	12.7
	Geometric mean	259	271
Source: Appendix 16.2.6			

Cmax (mIU/L)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	62.8 (13.4)	58.9 (17.7)
	Median	66.0	56.7
	Q1, Q3	51.6, 72.2	47.5, 64.2
	Min, Max	38.0, 83.5	31.3, 102
	CV(%)	21.3	30.0
	Geometric mean	61.4	56.7
2	No. of obs.	11	11
	Mean (sd)	58.8 (17.4)	61.7 (12.7)
	Median	52.0	62.3
	Q1, Q3	43.7, 68.1	53.5, 67.9
	Min, Max	41.0, 90.5	45.2, 87.7
	CV(%)	29.6	20.7
	Geometric mean	56.7	60.5
Total	No. of obs.	22	22
	Mean (sd)	60.8 (15.3)	60.3 (15.1)
	Median	60.3	57.6
	Q1, Q3	49.0, 70.4	53.5, 67.3
	Min, Max	38.0, 90.5	31.3, 102
	CV(%)	25.2	25.1
	Geometric mean	59.0	58.6
Source: Appendix 16.2.6			

AUC(0-inf) (hr*mIU/L)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	272 (47.6)	280 (38.8)
	Median	275	274
	Q1, Q3	231, 304	243, 317
	Min, Max	218, 368	230, 342
	CV(%)	17.5	13.9
	Geometric mean	269	278
2	No. of obs.	11	11
	Mean (sd)	259 (34.3)	313 (108)
	Median	257	298
	Q1, Q3	223, 288	240, 329
	Min, Max	219, 321	232, 616
	CV(%)	13.2	34.4
	Geometric mean	257	301
Total	No. of obs.	22	22
	Mean (sd)	266 (41.0)	297 (80.7)
	Median	257	287
	Q1, Q3	231, 288	243, 319
	Min, Max	218, 368	230, 616
	CV(%)	15.4	27.2
	Geometric mean	263	289
Source: Appendix 16.2.6			

t1/2 (hr)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	0.990 (0.409)	1.59 (0.596)
	Median	0.921	1.43
	Q1, Q3	0.745, 1.24	1.21, 2.23
	Min, Max	0.383, 1.88	0.798, 2.81
	CV(%)	41.3	37.6
	Geometric mean	0.917	1.50
2	No. of obs.	11	11
	Mean (sd)	1.13 (0.422)	1.95 (2.68)
	Median	1.03	1.16
	Q1, Q3	0.779, 1.37	0.655, 1.58
	Min, Max	0.502, 1.99	0.475, 9.90
	CV(%)	37.4	137.5
	Geometric mean	1.05	1.29
Total	No. of obs.	22	22
	Mean (sd)	1.06 (0.412)	1.77 (1.90)
	Median	0.980	1.35
	Q1, Q3	0.760, 1.34	1.03, 1.58
	Min, Max	0.383, 1.99	0.475, 9.90
	CV(%)	38.9	107.6
	Geometric mean	0.984	1.39
Source: Appendix 16.2.6			

tmax (hr)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	1.80 (0.835)	2.43 (1.41)
	Median	1.50	2.00
	Q1, Q3	1.25, 3.00	1.50, 2.50
	Min, Max	0.750, 3.00	1.25, 6.02
2	No. of obs.	11	11
	Mean (sd)	2.41 (1.23)	2.05 (0.779)
	Median	2.00	2.00
	Q1, Q3	1.50, 3.00	1.50, 2.50
	Min, Max	0.750, 5.02	1.25, 4.00
Total	No. of obs.	22	22
	Mean (sd)	2.10 (1.07)	2.24 (1.13)
	Median	2.00	2.00
	Q1, Q3	1.25, 3.00	1.50, 2.50
	Min, Max	0.750, 5.02	1.25, 6.02
Source: Appendix 16.2.6			

Pharmacokinetic biosimilarity assessment

Biosimilarity assessment was based on AUC(0-10 h) and Cmax. The results showed that Rechon Insulin fulfilled the criteria for biosimilarity with Humulin® Regular regarding the PK variables. The 90% CI for the ratio of the geometric mean value was within the pre-specified interval [0.8, 1.25] for both, the primary PK variable AUC(0-10 h) and the prioritised secondary PK variable Cmax. The ratios for the AUCs at the different time intervals were similar to the ratio for AUC(0-10 h). The range of the ratios was 0.909-0.968 for the other AUCs, compared to a ratio of 0.958 for AUC(0-10 h).

Bioequivalance assessment for primary and secondary PK variables, as well as the PK model output for Cmax and AUC(0-10 h) are presented in the following two tables.

Table 9: Pharmacokinetic analysis (PPS)

Variable[a]	Rechon Insulin Human Soluble (n=22)	Humulin® Regular (n=22)	Estimate for ratio between treatment	90 % confidence interval for ratio between treatments
$AUC_{(0\text{-}10\ h)}$	259	271	0.958	0.933 - 0.984
C_{max}	59	59	1.007	0.914 - 1.110
$AUC_{(0\text{-}2\ h)}$	72	75	0.968	0.880 - 1.064
$AUC_{(2\text{-}4\ h)}$	88	93	0.947	0.888 - 1.010
$AUC_{(0\text{-}4\ h)}$	162	170	0.954	0.896 - 1.015
$AUC_{(4\text{-}10\ h)}$	88	92	0.952	0.860 - 1.054
$AUC_{(0\text{-}\infty)}$	263	289	0.909	0.864 - 0.958
t_{max}	2.00	2.00	-	-
$t_{1/2}$	1.06	1.77	-	-

a Geometric mean value of AUC (h*mIU/L) and C_{max} (mIU/L), ratio between treatments and 90% CI for the ratio, median value of t_{max} (h) and arithmetic mean value of $t_{1/2}$ (h)

Source: Table 14.2.1.1 and Table 14.2.2.2

Distribution

Not applicable.

Elimination

See presented results regarding elimination half-life in section regarding bioequivalence.

Dose proportionality and time dependencies

Not applicable.

Special populations

Not applicable.

Pharmacokinetic interaction studies

Not applicable.

Pharmacokinetics using human biomaterials

Not applicable.

3.3.1.3. Pharmacodynamics

Pharmacodynamics were evaluated in a PK/PD combined single-centre, double-blind, randomised, single-dose, 2-period, crossover study using the euglycaemic clamp technique in healthy male and female subjects. The primary PD parameter was AUC for GIR from time zero to 10 h (AUC-GIR(0-10 h)) and secondary PD parameters were Maximum GIR (GIRmax), time to GIRmax (GIR-tmax), AUC-GIR(0-2 h), AUC-GIR(2-4 h), AUC-GIR(0-4 h) and AUC-GIR(4-10 h) for Insulin Human Rechon as compared to Humulin® Regular.

For further details on Study RCT-001, please refer to the above section regarding Bioequivalence.

Mechanism of action

The prime activity of insulin is the regulation of glucose metabolism. Its blood glucose lowering effect 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 Human Rechon is a fast-acting regular human insulin developed as 100 units/ml solution for subcutaneous injection in the upper arms, thighs, buttocks or abdomen using a reusable pen.

The euglycaemic hyperinsulinaemic clamp technique assesses the action of insulin by the required glucose infusion rate to maintain a pre-defined blood glucose concentration after injection of insulin. PD endpoints in study RCT-001 were obtained with a 10-hours euglycaemic manual clamp study after a 0.3 IU/kg subcutaneous injection of Insulin Human Rechon (test) and Humulin® regular (reference).

Primary and secondary pharmacology

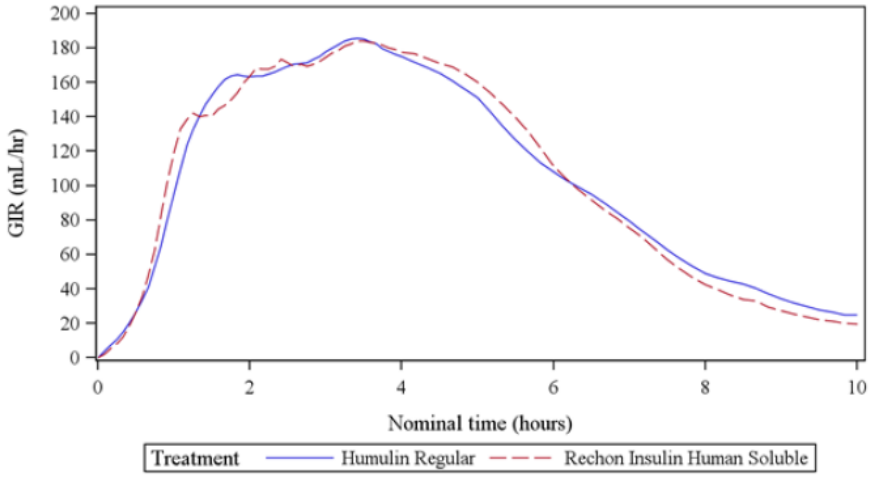
Study design and methods are described in more detail and are critically assessed in the above section regarding Bioequivalence (3.3.1.2.). All 22 subjects were included in the FAS, PPS and safety sets with no subject being excluded from PD analyses.

The PD of Insulin Human Rechon and Humulin® Regular was measured using a manual euglycaemic clamp technique. Analyses of blood glucose concentrations were performed continuously during the clamp study. An intravenous infusion of glucose (200 mg/mL) was adjusted to maintain the subject's plasma glucose at a target level of 6 mmol/L and the GIR was recorded. For details regarding PD methods, please refer to above section regarding Bioequivalence (3.3.1.2.).

Pharmacodynamic results

Mean GIR-time profiles from smoothed data as well as individual GIR plots were provided as overlay plot for each treatment. The mean GIR versus time curves (smoothed data) were very similar for Insulin Human Rechon and Humulin® Regular, while the median GIR-tmax was slightly longer for Humulin® Regular (3.2 h) than for Insulin Human Rechon (2.8 h). The mean GIR values (mL/hr) versus time (based on smoothed data) are shown in the figure below. Additionally, summary statistics for all PD endpoints are provided as depicted in the following tables.

Figure 9: Mean GIR curves based on smoothed data versus time (PPS)



Source: Figure 14.4.8

Table 10: Summary of pharmacodynamics variables (smoothed data; PPS)

AUC-GIR(0-10 h) (hr*mL/hr)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	1121 (304)	1066 (332)
	Median	1058	993
	Q1, Q3	841, 1260	799, 1394
	Min, Max	715, 1784	636, 1671
	CV(%)	27.1	31.1
	Geometric mean	1086	1020
2	No. of obs.	11	11
	Mean (sd)	993 (371)	1036 (242)
	Median	878	994
	Q1, Q3	676, 1398	822, 1227
	Min, Max	522, 1636	683, 1506
	CV(%)	37.3	23.4
	Geometric mean	932	1011
Total	No. of obs.	22	22
	Mean (sd)	1057 (337)	1051 (284)
	Median	1045	994
	Q1, Q3	782, 1260	822, 1263
	Min, Max	522, 1784	636, 1671
	CV(%)	31.9	27.0
	Geometric mean	1006	1015
Source: Appendix 16.2.6			

GIRmax (mL/(hr)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	249 (85.0)	201 (49.1)
	Median	243	173
	Q1, Q3	177, 326	165, 257
	Min, Max	130, 391	140, 278
	CV(%)	34.1	24.4
	Geometric mean	236	196
2	No. of obs.	11	11
	Mean (sd)	212 (99.2)	219 (75.3)
	Median	188	208
	Q1, Q3	139, 273	162, 266
	Min, Max	131, 445	110, 389
	CV(%)	46.8	34.4
	Geometric mean	195	208
Total	No. of obs.	22	22
	Mean (sd)	230 (92.1)	210 (62.7)
	Median	203	201
	Q1, Q3	147, 294	165, 257
	Min, Max	130, 445	110, 389
	CV(%)	40.0	29.8
	Geometric mean	214	202
Source: Appendix 16.2.6			

GIR-tmax (hr)

Day		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
1	No. of obs.	11	11
	Mean (sd)	2.38 (1.49)	3.19 (0.715)
	Median	1.67	3.25
	Q1, Q3	1.17, 4.08	2.67, 4.00
	Min, Max	1.00, 5.00	1.83, 4.17
2	No. of obs.	11	11
	Mean (sd)	3.50 (1.26)	3.69 (2.42)
	Median	3.25	3.17
	Q1, Q3	2.28, 4.33	2.17, 4.00
	Min, Max	2.00, 5.83	1.83, 10.0
Total	No. of obs.	22	22
	Mean (sd)	2.94 (1.46)	3.44 (1.76)
	Median	2.83	3.21
	Q1, Q3	1.67, 4.08	2.33, 4.00
	Min, Max	1.00, 5.83	1.83, 10.0
Source: Appendix 16.2.6			

Pharmacodynamic biosimilarity assessment

The results from the primary PD analysis were presented together with the results from analysis of the secondary variables (smoothed data). The 90% CI for the ratio of the geometric mean value was within a pre-specified bioequivalence acceptance range [0.8, 1.25] for both the primary PD variable AUC-GIR(0-10 h) and the prioritised secondary PD variable GIRmax.

An addendum, dated on 06 March 2015, was written as an addition to the PD analyses described in the clinical study report for study RCT-001. In the protocol and statistical analysis plan for the study, a 90% CI for the ratio of the two treatment means was chosen for both PD and PK parameters. However, in accordance with the recent guideline (EMEA/CHMP/BMWP/32775/2005_Rev. 1), the 90% CI of the ratio test/reference should be determined for the primary PK endpoints AUC and Cmax, but calculation of the 95% CI is required for the primary PD endpoints AUC-GIR and GIRmax. The PD analyses described in the study report were therefore repeated with a 95% CI for the ratio between the two treatments (using the same acceptance limits [0.8, 1.25]). As depicted in the table below, Insulin Human Rechon fulfilled the criteria for biosimilarity with Humulin® Regular, i.e. the 95% CIs for AUC-GIR(0-10 h) and GIRmax were within the pre-specified interval [0.8, 1.25].

In addition, the model output from PD analyses for AUC-GIR(0-10 h) and GIRmax were presented and are depicted below.

Table 11: Pharmacodynamic analyses (smoothed Data; PPS)

Parameter	Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)	Estimate for ratio between treatment	95 % confidence interval for ratio between treatments
$AUC\text{-}GIR_{(0\text{-}10\ h)}$	1006	1015	0.991	0.922 - 1.064
GIR_{max}	214	202	1.062	0.960 - 1.176
$AUC\text{-}GIR_{(0\text{-}2\ h)}$	154	158	0.980	0.827 - 1.162
$AUC\text{-}GIR_{(2\text{-}4\ h)}$	320	331	0.967	0.878 - 1.066
$AUC\text{-}GIR_{(0\text{-}4\ h)}$	479	491	0.976	0.884 - 1.076
$AUC\text{-}GIR_{(4\text{-}10\ h)}$	498	490	1.015	0.920 - 1.121

Table 12: Model output from pharmacodynamic analysis (PPS)

Parameter	Residual CV (%)	p-value sequence effect	p-value period effect
AUC-$GIR_{(0\text{-}10\ h)}$	11.4	0.567	0.029
GIR_{max}	16.3	0.376	0.200
Source: Appendix 16.2.6			

Clamp quality

Mean glucose levels during the euglycaemic clamp studies were summarised for the two IMPs and are displayed in the table below. The data showed that the mean glucose level was approximately 6.0 mmol/L for both IMPs over the clamp duration and that the intra-individual CVs on average were close to 10 %. Results from the glucose measurements and CVs during the euglycaemic clamp studies were provided as list by subject and sample time point.

Table 13: Summary of individual glucose levels by treatment (PPS)

		Rechon Insulin Human Soluble (n=22)	**Humulin® Regular (n=22)**
Mean Glucose level (mmol/L)	No. of obs.	22	22
	Mean (sd)	6.04 (0.0942)	6.04 (0.0804)
	Median	6.03	6.06
	Q1, Q3	5.97, 6.09	6.00, 6.10
	Min, Max	5.84, 6.21	5.86, 6.21
Coefficient of variation (CV) for Glucose level	No. of obs.	22	22
	Mean (sd)	10.4 (2.98)	9.51 (1.59)
	Median	10.4	9.15
	Q1, Q3	7.92, 12.2	8.17, 10.5
	Min, Max	6.73, 19.2	6.65, 12.5

CV = 100 * sqrt(exp(sd*sd) - 1) where sd is the standard deviation for logarithmic data

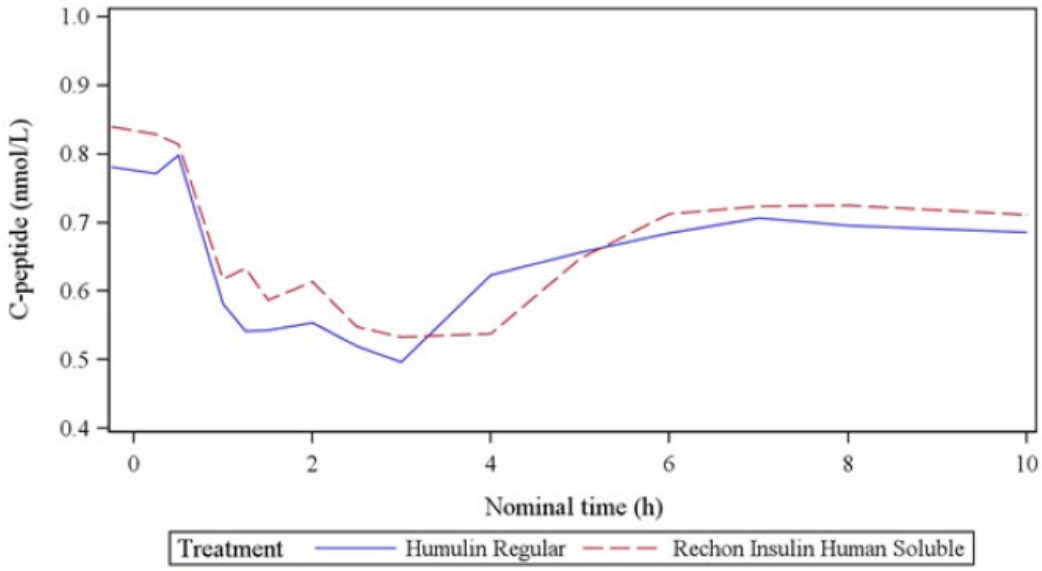
Source: Table 14.2.2.3

C-peptide plasma concentrations

C-peptide plasma concentrations per time point were provided as list by subject and as summary.

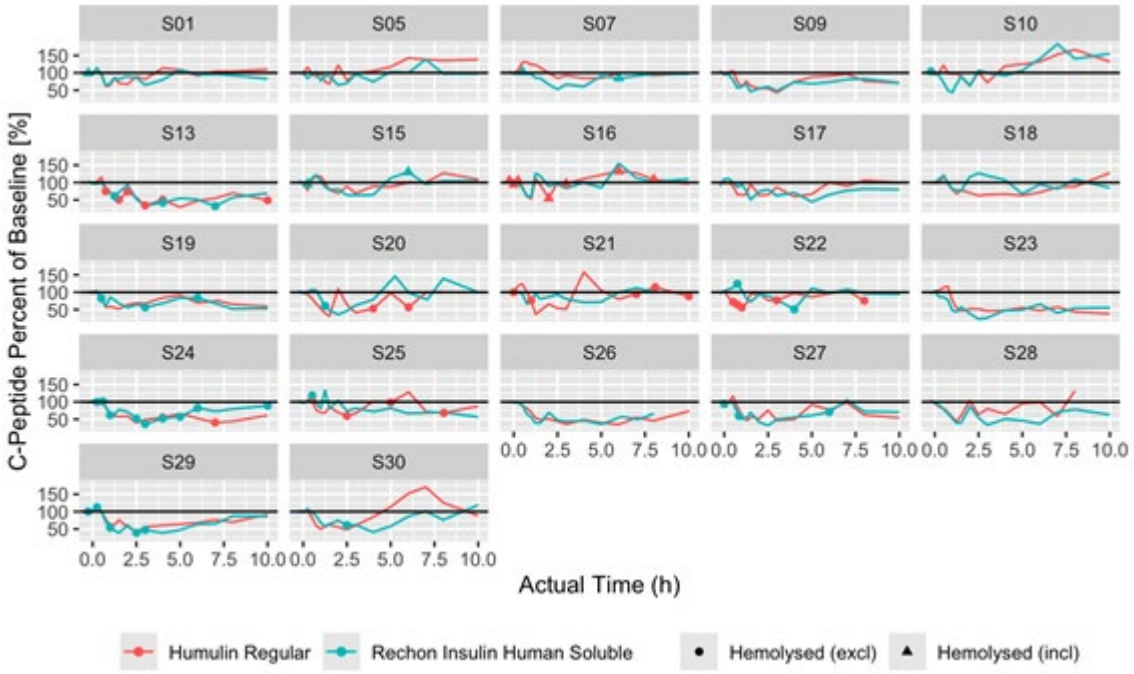
The mean C-peptide plasma concentration decreased in a similar way after administration of both IMPs showing that the endogenous insulin production was decreased upon IMP administration. After 6 hours and until the end of the 10-hours clamp period, the C-peptide plasma concentration had returned to approximately the same value as before the IMP administration.

Figure 10: Mean C-peptide concentrations versus time (PPS)



Source: Figure 14.4.6

Figure 11: Individual time course of C-Peptide as percentage of individual baseline



Intra-subject variability of primary measures

Table 14: Intra-subject variability (CV%)

	Insulin PK		GIR PD	
	AUC*	C_{max}	AUC*	GIR_{max}
RCT-001	5.1	18.9	11.3	16.3

* AUC_{0-10}.

3.3.2. Discussion on clinical pharmacology

Study RCT-001

Study RCT-001 was conducted as randomised, double-blind, single dose, two-period, crossover, 10-hour euglycaemic clamp study to assess PK/PD biosimilarity between Insulin Human Rechon (test) and Humulin® Regular (reference). The overall study design adheres to the current EMA guideline (EMEA/CHMP/BMWP/32775/2005_Rev. 1) and is thus, acceptable. The wash-out between the two periods of treatment was only 24 hours but is considered acceptable due to the short half-life of the product (<2 hours) and as no carry-over effect was identified.

Study Population, treatments and study procedures

The GL (EMEA/CHMP/BMWP/32775/2005_Rev. 1) recommends including only men as the study population should be homogenous and insulin-sensitive to best detect potential product-related differences. In study RCT-001 also female subjects were included, which is however accepted as due to the cross-over study design subjects act as their own control and thus no concerns regarding the conclusion on biosimilarity arise. Inclusion and exclusion criteria were generally acceptable. Also the enrolment of healthy volunteers appears principally acceptable, as lower intra-individual variability is to be expected compared to patients with type 1 diabetes. However, healthy individuals do have sufficient endogenous insulin, which might interfere with PK and PD measurements. The applied dose of 0.3 IU/kg is in line with guidance and principally considered sufficient to suppress endogenous insulin in the fasting state. Furthermore, C-peptide was measured to estimate the secretion of endogenous insulin during the study. The reference product holds a valid licensure in Sweden and is considered adequate. Blood glucose concentrations are recommended to be clamped at or below the subjects fasting glucose or at 4.4-5.6 mmol/L in healthy volunteers (see guideline EMEA/CHMP/BMWP/32775/2005_Rev. 1).

The applicant has however applied a target glucose level of 6 mmol/L in this study that is above this recommended range. Blood chemistry analysis results of enrolled subjects show that mean screening- (day -21 to day -1) and follow up- (day 6-8) visit glucose values were <5 mmol/L (screening mean 4.6 with min: 4.1 and max: 5.2 mmol/L; follow-up mean 4.72 with min: 4.1 and max: 5.3mmol/L), even though no overnight fasting was required for these visits according to study plan. Also, glucose measures starting 120 minutes before the clamp start are mostly <6 mmol/L (range: min 4.4mmol/L, max: 6.8mmol/L; N=29 <6mmol/L, N=4 ~6mmol/L, N=8 >6mmol/L) and were adjusted to achieve the intended clamp level of 6 mmol/L at pre-dose. Reasons for the apparently higher fasting glucose level in comparison to the screening/follow-up glucose levels in unclear. Notably, no data on C-peptide and/or insulin are provided before glucose infusions started or for the time interval starting from 120 minutes before treatment, which would allow for some insight in endogenous insulin levels pre-treatment. It is acknowledged that pre-treatment with glucose in RCT-001 stabilized to the target level within the first hour (-120 to -60 min), reducing variability and glucose control was achieved during the baseline period (-60 to -0 min). In that time the clamp target was maintained by a stable glucose infusion rate in most patients. However, glucose levels seem to fluctuate around the clamp level throughout the pre-treatment period (also during the phase of -60 to 0 min with stable GIR), indicating that endogenous insulin continuously acts on (elevated) glucose levels, which are then compensated by continuous glucose infusions.

It is concluded that glucose infusions that were required to adjust glucose levels to the clamp target were stimulating endogenous insulin release, at least in most participants. This impression is further supported by mean C-Peptide levels at baseline, which appear higher (around 0.8nmol/L) compared to expected C-Peptide levels during fasting glucose levels (rather below 0.6nmol/L would be expected at fasting). C-peptide suppression in relation to pre-dose levels upon study insulin administration is

acknowledged for both products 1 to 4 hours after insulin administration. However, suppression seems insufficient as the mean level stays above 0.5nmol/L for all sampling time points, a level just below the expected fasting level. This indicates a potentially incomplete suppression of endogenous insulin secretion during the study.

Hence, the elevated glucose clamp target concentration (with respect to fasting glucose levels) expectedly leads to an increase of endogenous insulin while adjusting to the target glucose level during clamp, before the exogenous insulin is given. The chosen target glucose level is not optimal and the lack of C-peptide and/or insulin measures before and from beginning of glucose adjustments is an additional drawback. The exact level of endogenous insulin contained in the PK/PD measures performed is difficult to determine. Importantly, it is also unknown whether the influence was balanced or uneven between the proposed biosimilar and the reference insulins. As a consequence, the impact of the elicited secretion of endogenous insulin on the ratios of Insulin Rechon and Insulin Humulin is unknown and renders the equivalence assessment on the exogenous insulin from the PK/PD measures is considered uninterpretable. As this is essential for the conclusion on biosimilarity, the available data from study RCT-001 are presently not sufficient to establish PK/PD equivalence of insulin Rechon versus the reference. Compelling evidence would be required to demonstrate equivalent PK/PD (**MO**).

Also, blood sampling frequency for C-peptide and insulin measures appears very sparse around Tmax, which hampers a robust estimation of Cmax (labelled as "prioritised" secondary endpoint by the applicant, but as per guideline to be considered a variable of primary relevance). The applied sampling frequency was every 15 minutes for the first 1.5 hours, then every 30 minutes for the following 1.5 hours (i.e. 3 hours after start) and every 60 minutes until end of study (i.e. 10 hours after start). A sampling frequency of 10-15 minutes around Tmax would be considered adequate to estimate Cmax with sufficient precision. However, with Tmax being estimated after around 2 hours, the applied sampling frequency of 30 minutes is considered too sparse to provide reliable estimation of Cmax. Potential differences in insulin concentrations between both products with respect to Tmax and Cmax might have been missed with the applied sampling frequency. Thus, assessment of one of the most important measures for biosimilarity appears compromised by the applied study design. No rationale was provided for the decreased sampling frequency after 1.5 hours post-dosing, while knowledge on Humulin R and the expected tmax was available. However, it is acknowledged that the impact of the wider sampling frequency on the accuracy of Cmax evaluation is not significant due to relatively stable levels around Cmax and the absence of steep fluctuations around the peak insulin concentration. The blood insulin concentration profile from study RCT-001 indicate "stable" levels between 1.5 and 2.5 hours, followed by a steady decline. Notwithstanding, the decreased sampling frequency around tmax is inherently linked with a decreased accuracy of Cmax evaluation and poses an additional source of uncertainty. In summary, the applicant has not provided reasoning for the decreased sampling frequency and the sampling frequency around tmax is suboptimal. Nevertheless, the levels around Cmax are considered sufficiently stable such that Cmax is accepted as sufficiently covered, though Tmax may not be fully covered and this aspect remains as uncertainty.

Blood sampling frequency for glucose measurements and other study procedure descriptions appear acceptable.

Endpoints

The primary PK and PD endpoints (AUC0-10 and GIR-AUC0-10) are acceptable to address the primary objective (i.e. to demonstrate similar PK and PD between Insulin Human Rechon and Humulin®). However, according to the GL (EMEA/CHMP/BMWP/32775/2005_Rev 1), Cmax and GIRmax should be defined as Co-primary endpoints for rapid-/short-acting insulins, but were specified as secondary endpoints in the study protocol. As Cmax and GIRmax are within the biosimilarity acceptance range, no concern is raised. Other secondary PK and PD endpoints were adequately specified.

Assays

The applicant used commercial immunoassay kits for in vitro detection and quantification of serum insulin and serum C-peptide. It is acknowledged that both assays are commercial assays and that the assays were run in a central laboratory. Thus, the assays used are well known and documentation from the manufacturer justifies the quality of the assay with regards to its components. The applicant reports some laboratory statements on in-house assay performance. Reference intervals for the C-Peptide assay were confirmed with healthy subjects and a precision was determined with CV of 7.1% at the level of 0.95 nmol/L. Notably, also values >0.95 nmol/L of C-Peptide were recorded during study RCT-001, which leaves uncertainty regarding assay precision at these values. The precision of the insulin assay was concluded ≤5% CV, also with a concentration that is above levels actually measured during study RCT-001. Calibration was done with international standards, which is acknowledged. In summary, the lack of a well-documented confirmation regarding in-house validation of assay performance is critically noted, especially considering the uncertain precision of higher C-Peptide levels. Considering that used assays are widely used commercial assays, no further concern is raised, but an uncertainty remains that is considered in the assessment.

The commercial device that was used for glucose measurements is factory calibrated and is validated by the manufacturer. The accuracy of all systems used in study RCT-001 was confirmed by glucose reference samples before study start. The variability seems acceptable, and also the reported maximum deviations do not give rise to concern

Methodology

The final sample size of 22 was adequate for the AUC(0-10h) PK endpoint. For PD variables, however, given the broader 95% CI test/reference ratio to be contained in the 80-125% acceptance range, more than 11 subjects per period group would have been reasonable. Also no sample size calculations were provided for Cmax and GIRmax, which are considered primary. However, this will not further be pursued, as the results are prevailing.

No concerns are raised regarding the randomisation or blinding procedure. However, a data review was performed, prior unblinding, in which, among other things, decisions were made about the duration of the baseline period for GIR and the parameters to be set for the LOESS smoothing. Upon request, the applicant justified the choice of the baseline period for GIR due to a decreased variability in blood glucose levels and improved stability in GIR 1 hour prior the IMP administration with respect to the 2-hour time point prior dosing. Thus, the chosen period of 60 minutes prior IMP administration is acceptable. Furthermore, although the smoothing method is frequently applied and accepted by EMA guidance in order to evaluate GIRmax and tmax, the determination of the smoothing parameters raises concerns since the criteria according to which the choice for the smoothing parameters were made are not adequately described. Therefore, it cannot be ruled out that a smoothing factor has been chosen based on its ability to let the data appear more similar. In order to examine the impact of the smoothing factor of 0.2, sensitivity analyses were requested for GIRmax and GIR-tmax using several smoothing parameters. Baseline GIR was given 10 times the weight of the other data points during GIR smoothing, which is considered reasonable as baseline GIR represents an average of multiple pre-dose measurements.

In the statistical analysis models, fixed effects were used for sequence, period and formulation. A random effect was used for subject within sequence but given the design of the study including one observation per period, this is equivalent to the use of fixed effects for subject as recommended by the EMA Guideline on Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **). No concerns are raised with respect to the TOST procedure in general or the 80-125% acceptance range.

For the statistical analyses baseline-corrected concentration for the PK parameter were used. Thereby negative serum concentrations after baseline subtraction were either set to zero or omitted from the analysis. The same baseline-correction was applied in the calculation of the PD parameters from the smoothed GIR data. This baseline-correction is seen critical and additional analyses using an ANCOVA based on uncorrected values (i.e. all measured values without subtracted baseline mean and without smoothing of GIR values) as dependent variable and with baseline values as covariates were requested for the primary PK and PD endpoints Cmax, AUC(0-10h), GIRmax and AUC-GIR(0-10h).

Two study amendments were conducted, the information for both protocol amendments were sufficiently outlined and no concerns arise. There were no study drop-outs and discontinuations reported and no subjects were excluded for PK and PD analysis.

Study conduct

The demographic characteristics were balanced between treatment sequence. It is stated that a few minor protocol deviations are documented in the Declaration of Database Lock. Upon request, the Declaration of Database Lock document and details of protocol deviations were provided. There were 15 protocol deviations reported for 12 out of 22 subjects (54 %). Out of the 15 protocol deviations, 9 deviations concerned PK and PD evaluations. It is unclear why the error in glucose infusion rate adjustment for one subject was not reported as protocol deviation. Notwithstanding, the applicant concluded that this administration error had an impact on PD assessments. Indeed, removal of the erroneously high value for this subject from requested analyses on raw unsmoothed GIR affected the result for GIRmax (see below "Pharmacodynamics"). Regarding the occasions of subcutaneous glucose infusion, the provided listing states that *some* glucose infusion was received subcutaneously but the number of occasions or duration of this deviation is not further specified. Affected subjects were one at dose 1 (Rechon) and another at dose 2 (Rechon). Reason and consequences of subcutaneous glucose are not clear, but consequences appear not to affect the general conclusion.

Pharmacokinetics

Summary statistics and per subject listings for PK variables were provided and presented results largely appear comparable between both insulin treatments. However, the terminal half-life (t ½) is lower for Insulin Human Rechon (mean (SD): 1.06h (0.4); median: 0.98h) than for Humulin® (mean (SD): 1.77h (1.9)). In addition, the CV% for t½ is substantially higher in Humulin treatment period 2 (137.5%) in comparison to period 1 (37.6 %) and Insulin Human Rechon (41.3 % period 1, 37.4 % period 2). This imbalance and variability may have been driven by a single subject in one period, which displayed an insulin t1/2 of 9.9h for Humulin but 1.49h for Rechon. The same subject also had a substantial increase in C-peptide levels directly after the injection of Humulin insulin (from baseline around 1.3 to 1.7 back to 1.3 mmol/L within the first 2h), indicating the release of endogenous insulin in this period, as well as increasing GIR until end of measure after 10h. No protocol deviations, safety events, concomitant medication were evident for the subject that could influence the half-life and the extended half-life did not translate into any unusually extended PD effect. Thus, no clear reasons for the high value and no immediate consequences are evident. As a consequence, it is not fully clear whether the measure can indeed be considered an outlier, which would justify an exclusion. Still, in order to gain further insight the applicant reported half-life calculations for Humulin with this subject excluded upon request. After exclusion, the mean, median and geometric mean half-life of Humulin are slightly lower (from 1.78h, 1.35h and 1.39h to 1.38h, 1.27h and 1.26h, respectively), but are still clearly higher (around +30%) compared to Rechon Insulin (1.06h, 0.98h and 0.984h, respectively). The half-life becomes a bit more comparable between treatment arms when following a C-Peptide corrected PK analysis on top of the subject exclusion for the Humulin PK (mean, median and geometric mean for Humulin: 1.22h, 1.09h and 1.1h compared to Rechon Insulin: 1.08h, 1.10h and 0.992h, respectively), which indicates a possible interference of endogenous insulin on the estimation of the

half-life. It is unclear whether the remaining difference is indeed due to normal variability. Claimed limitations in precisely estimating terminal elimination phases could have been overcome by a more suitable study design. No statistical hypothesis testing was conducted for half-life estimations between treatments. Thus, it cannot be excluded that the proposed biosimilar Insulin Human Rechon might have a slightly shorter half-life compared to the originator Humulin Insulin. This observation remains as an uncertainty regarding the proposed biosimilarity of both insulin products.

In total, 65 haemolytic blood samples were identified. After blinded data review it was decided that results from the haemolysed blood samples for insulin determinations should be excluded from calculation of PK variables as haemolysis seems to interfere with insulin plasma measurements (i.e. result in lower insulin concentrations). The majority of subjects had samples taken that were haemolysed (15 of 22). In total, 8.8% of all samples provided for insulin analysis were haemolysed samples, 65 haemolytic blood samples were identified (of in total 742 samples) and 53 of these samples were excluded for PK data analysis. It is unclear why 12 samples were not excluded, but a sensitivity analysis with removal of all haemolysed samples did not result in substantial differences regarding PK parameters and conclusions on bioequivalence. Reasons for haemolysed samples and potential reasons for clustering of haemolysed samples in specific subjects do not appear evident, but clustering of haemolysed samples was balanced between treatment arms (5 Humulin R and 4 Rechon insulin PK profiles had 3 or more haemolysed samples). Notably, values from haemolysed samples were already flagged in the Bioanalysis report from the central laboratory where insulin assays were performed, which is reassuring concerning a potential data driven selection. Exclusion appears justified given the bad quality of samples, residual uncertainty on study conduct nevertheless remains.

The 90% CI of the geometric mean ratios for the primary PK endpoint, AUC(0-10h), as well as for the prioritized secondary endpoint, Cmax, and all other secondary endpoints are within pre-defined bioequivalence acceptance range (0.8-1.25): 90% CIs 0.933-0.984 for AUC(0-10h) and 0.914-1.110 for Cmax. No statistically significant period- and/or sequence-effects were observed for PK variables. The requested sensitivity analyses for AUC(0-10h) and Cmax without baseline-correction but including the baseline concentration as covariate in the ANCOVA model gave similar 90% confidence intervals: 0.938-0.989 for AUC(0-10h) and 0.930-1.107 for Cmax. Thus, based on the provided results, PK biosimilarity between Rechon insulin (test) and Humulin insulin (reference) was demonstrated with available data. However, deficiencies in the study design are critically noted with respect to clamp glucose target levels and sampling intervals (see above).

Pharmacodynamics

A manual clamp technique was conducted to assess glucose infusion rate (GIR) as a PD measure. The euglycaemic clamp technique is considered the most suitable available method to assess PD of insulin (insulin action). The overall study design is acceptable and in line with the GL (EMEA/CHMP/BMWP/32775/2005_Rev. 1). An algorithm for GIR adjustments was developed during the pilot study and was further optimized during the main study. Furthermore, it is stated that some subjects required GIRs adjustments outside the ranges of the algorithm. Thus, the pre-specified algorithm for GIR adjustment was overall not sufficient to cover the entire range of blood glucose fluctuations in the clamp study. Details regarding timing and character of the algorithm optimization as well as for the reported range escalations with respect to amplitude, number of affected subjects, treatment period and applied GIR adjustments were provided upon request. The applicant clarified that these optimizations were made throughout the entire clamp period and were based on glucose measurements and operator judgement. Hence, the extent of applied infusion rate adjustments outside the algorithm range was not standardized and thus, are an additional source of variability. Based on provided graphical presentation of individual blood glucose levels, upper and lower glucose levels covered by the GIR algorithm were exceeded in almost every subject, with seemingly more than half of the subjects requiring multiple additional adaptations and some (roughly 7 out of 22) having blood

glucose levels of almost 8 mmol/L (i.e. 2 mmol/L above the clamp target) at some point during the clamp period. One draw-back of sub-optimal algorithms for GIR adjustments is often reflected in the absorption phase of fast acting insulins. In case of a too slow adaptation of the GIR to compensate for the onset of insulin action, a drop in blood glucose levels occurs followed by an overshoot of blood glucose levels due to a delayed GIR adjustment. This observation is indeed apparent for some subjects during the earlier phase of the clamp period, but not exclusively. In case of manual clamps, the less frequent glucose measurements with respect to automated clamps poses another challenge. Notwithstanding, the individual variability in blood glucose level is acknowledged. It is also agreed that observed fluctuations seem to overall follow similar trends within-subject (i.e. between treatment) reflecting the individual insulin sensitivity and glucose requirements, while exceptions with opposite trends within-subject are also apparent. The applicant states that the manual adjustments were balanced between the test and reference insulin treatments. To better evaluate this statement, the applicant was asked to provide summarized data on occurrences requiring GIR adjustments. There were in total 974 and 953 out-of-algorithm changes in all subjects (n = 22) during the Insulin Rechon and Humulin period, respectively. The number of events was approximately balanced between treatments when considering the entire clamp period (0-10 h) and certain time intervals (0-2 h, 2-4 h, 4-10 h). Hence, except for some numerical differences concerning changes by >25%, no systematic treatment bias is apparent. However, the overall number of not pre-specified changes appears high with respect to the total number of GIR measurement/adjustment time points (measurement at time 0, then every 5 minutes up to 4 hours post-IMP administration, every 10 minutes until the end of the clamp resulting in roughly 1870 measurements per study period for all 22 subjects). The impact of such deviations on biosimilarity assessment is uncertain. As the undertaken GIR adjustments informing primary PD parameters to a certain degree lack standardization, this observation poses an additional source of uncertainty affecting a large number of measurement time points. Therefore, this issue remains as uncertainty that is considered in the assessment.

PD Results

The mean GIR-time curves (smoothed data) were very similar for Insulin Human Rechon and Humulin® Regular. The mean AUC-GIR(0-10 h) was comparable for Insulin Human Rechon (1057 h*mL/h, range 522-1784) and for Humulin Insulin (1051 h*mL/h, range 636-1671). Mean GIRmax values were also similar with 230 mL/h (range 130-445) for Insulin Human Rechon and 210 mL/h (range 110-389) for Humulin Insulin. GIR-tmax mean and median values indicate a later GIRmax for Humulin Insulin (3.2 h [median] and 3.44 h [mean], respectively, range 1.83-10.00) than for Insulin Human Rechon (2.8 hr [median] and 2.94 [mean], respectively, range 1.00-5.83). This imbalance appears to be largely driven by a single subject in period 2. The sensitivity analyses using a smoothing factor of 0.05, 0.1 and 0.15 revealed no systematic dependence between the results for GIR-tmax and the amount of smoothing, which is reassuring. Initially the applicant has planned with a 90% CI, as presented in the study report, but submitted results for a 95% CI for the ratio between the two treatments (using the same acceptance limits [0.8, 1.25]) as addendum to the study report. This is supported and in line with EMA GL EMEA/CHMP/BMWP/32775/2005_Rev. 1.

Solely the 95% CIs are assessed in the following, as those are relevant for the PD endpoints (i.e. AUC-GIR and GIRmax). The 95% CI of the geometric mean ratio for the primary PD variable AUC-GIR(0-10 h) was 0.922 - 1.064 and is thus contained within the pre-specified bioequivalence acceptance range [0.8, 1.25]. This is in line with AUC(0-10) of insulin PK results. Also the 95% CI of the prioritized secondary EP GIRmax with 0.960 - 1.176 is within this bioequivalence acceptance range. A statistically significant period effect for AUC-GIR(0-10 h) (p-value: 0.029) was identified with a consistently higher GIR in treatment period 1. However, it is to be noted that no concern regarding biosimilarity arises from the observed period effect in this cross-over study as both treatments were equally affected and especially as the washout time of 24h is deemed long enough considering the short elimination half-life

of <2h. The requested sensitivity analysis for GIR-AUC(0-10h) without baseline-correction and without smoothing but including the baseline value as covariate in the ANCOVA model gave similar results as the primary analysis. However, for GIRmax the sensitivity analysis on the raw data gave a 90% confidence interval of 0.979-1.253, implying that also the 95% confidence interval (not provided) exceeds the acceptance range of 0.8-1.25. This is in line with the results from the sensitivity analyses exploring different choices of smoothing factors for GIRmax, where the 95% confidence intervals was contained within the acceptance range of 0.8-1.25 for a smoothing factor of 0.15 but exceeded the equivalence range for a smaller smoothing factor of 0.05 or 0.1. This dependence of the results for GIRmax on the applied amount of smoothing is especially concerning as the choice of the smoothing factor was made during blinded data review. Part of the difference in GIRmax between the treatment arms observed with less smoothing might be explained by an error in clamp administration. For subject S10 the infusion rate of glucose was increased from 215.5 mL/h to 724 mL/h at minute 310 on the Rechon treatment day, although the glucose level was reported to be 7.1 mmol/L, which would have required a decrease of the GIR by 20% according to the algorithm for GIR adjustments. For the sensitivity analysis for GIRmax using the raw data without baseline correction, exclusion of the erroneously high value for one subject changed the results from not meeting the bioequivalence criteria to meeting the criteria. Excluding the erroneously high value for the subject is considered a reasonable approach as it approximates the preferable situation where the clamp was performed according to the protocol. Thus, the applicant was asked to present revised sensitivity analyses exploring a smoothing factor of 0.05, 0.10 and 0.15 when excluding the erroneously high value for the subject. These sensitivity analyses resulted in 95% confidence intervals lying within the acceptance range of 0.8-1.25 and thus resolve former concerns regarding specification of the smoothing factor during blinded data review. In conclusion, based on the provided results, biosimilarity between Insulin Human Rechon (test) and Humulin insulin (reference) would be demonstrated for all PD parameters, provided the results were acceptable given the concerns on the clamp level.

Clamp quality

Clamp quality measures were presented as summary statistics for glucose levels throughout the clamp period and CV for glucose levels within subjects (intra-subject CV). Considering reported mean and median levels of glucose, the clamp quality seems acceptable, as in line with the pre-defined clamp level (i.e. 6mmol/L). Mean and Median intra-subject CV for glucose levels is approximately 10%. However, it is unclear if the clamp quality definition was set out on beforehand in the protocol as the GL (EMEA/CHMP/BMWP/32775/2005_Rev. 1) recommends. The applicant compared upon request reported intra-subject CV of blood glucose during treatment with values reported in four publications where presented median %CV ranged approximately between 5-6%. Hence, the in study RCT-001 recorded median %CV of roughly 10% is 2-fold higher than values reported in the literature for other studies – independent of manual or automated clamp procedures. However, the clamp quality was overall comparable between study arms. While the quality of the glucose clamp in study RCT-001 was lower with respect to reported values in the literature, no immediate concerns derive from the higher CV% (compared to other studies).

C-Peptide

C-peptide levels were monitored throughout the clamp study, which is endorsed and according to current GL recommendations (EMEA/CHMP/BMWP/32775/2005_Rev. 1). This is especially critical as the study population included healthy volunteers. To address concerns pertaining to endogenous insulin release during the study, the applicant provided further presentation on individual C-peptide profiles as percentage of baseline to better estimate inter- and intra-individual trajectories, which is acknowledged. Mean C-peptide concentration profiles indicated C-peptide suppression upon study insulin administration, but notably, the mean level stayed above 0.5nmol/L for all sampling time points. Further, approximate baseline levels were reached after 6 hours for test and reference insulin

but also appeared not fully aligned between both study insulins. The provided percentage of baseline presentation shows that C-peptide reached above baseline levels at some point during the clamp periods in roughly half of the subjects. Thereby, not only a continuous rise in C-peptide was apparent, but also C-peptide "spikes" occurred. It can be agreed though that the C-peptide levels in the study population follow mostly intra-individual trends reflective of individual differences in insulin sensitivity and glucose metabolism. However, within-subject differences for the C-Peptide profile as well as the magnitude of C-peptide rise may differentially impact PK/PD measures. Thus, whether endogenous insulin suppression was sufficient to exclude potential confounding issues remains unclear as discussed below.

Individual variability in C-peptide levels, including physiological fluctuations, is acknowledged and thus, it is reasonable to assume that a rise in C-peptide may not necessarily always indicate glucose-stimulated insulin release. However, to which extent endogenous insulin post-dosing and threshold levels thereof may impact the interpretability of clamp study results seem not entirely clear. The applicant states, based on one literature report, that an increase in C-peptide greater than 50% with respect to baseline may be indicative of insufficient endogenous insulin expression potentially compromising the accuracy of PD results. Notably, the cited study is subject to several limitations including a small sample size. According to the applicant, a C-peptide increase above 150% of baseline was reached in three subjects. The applicant's conclusion that these observations were not impactful on study results is due to a lack of an apparent effect on insulin blood concentration curves. While this observation is agreed on, the applicant did not sufficiently consider all aspects. Observing an impact on PK parameters due to endogenous insulin may be hampered due to the short half-life of insulin (5 to 10 min) with respect to C-peptide (30 to 35 min) and thus, may not be captured by the applied sampling frequency. Still, the action of endogenous insulin release could translate into biological action reflected by GIR. Also, the definite effect on PK/PD measurements may depend on the absolute amount of released insulin. For instance, Heise et al propose that experiments with a C-peptide rise by >0.2 mmol/L from baseline should be excluded. Further, "C-peptide criteria" guiding the exclusion of subjects from PD analysis were employed in previous short-/rapid-acting insulin biosimilarity studies highlighting the overall concern pertaining to the impact of endogenous insulin on the interpretability of biosimilarity.

To reiterate, the variability of glucose metabolism and potential individual oscillations of endogenous insulin as well as assay variability are acknowledged, but in the light of identified study design issues, the unclear role of endogenous insulin release and cases of divergent profiles within subjects leave room for uncertainty pertaining to the sensitivity of the biosimilarity assessment. Even if not all, but only a handful of subjects are affected, this is considered to potentially impact study conclusions also bearing in mind the sample size of 22 subjects. These points considered, this issue is further pursued as part of the outstanding clinical **MO**.

Variability

Inter-individual CV(%)was somewhat higher for subjects treated with Rechon (AUC(0-10): 14.9%, Cmax: 25.2%, GIRmax: 40%, AUC-GIR(0-10): 31.9%) compared to Humulin (AUC(0-10): 12.7%, Cmax: 25.12%, GIRmax: 29.8%, AUC-GIR(0-10): 27%). Especially GIRmax shows a difference of >10% between treatments (above reported the arithmetic inter-individual variability, but results are largely comparable for the geometric CV%). This observation indicates that a more variable degree of glucose infusion was required across subjects to keep the target glucose level when treated with Rechon. Glucose infusion is only required for the clinical study setup in healthy subjects, but is indicative of insulin effects on blood glucose levels. Whether or to what extent endogenous insulin contributed to the more variable need for glucose when treated with Rechon is not clear. Thus, it cannot be excluded that the inter-subject variability is indeed higher for Insulin Human Rechon. This observation remains as an uncertainty regarding the proposed biosimilarity of both insulin products.

For sample size considerations the applicant assumed that the intra-individual coefficient of variation (CV) for AUC(0-10 h) is about 18% and estimated the intra-individual CV of the ln transformed AUC-GIR(0-10 h) to be 20%. The actual intra-subject variability was bit lower than anticipated for sample size considerations (CV% for Cmax: 18.9, AUC(0-10): 5.1%, AUC-GIR(0-10 h): 11.3% and GIRmax: 16.3%).

3.3.3. Conclusions on clinical pharmacology

In conclusion, PK/PD data do not indicate major imbalances between the reference product and the proposed biosimilar treatments from reported results on primary variables. Still, the applied target glucose clamp level of 6 mmol/L in study RCT-001 is above the actual fasting glucose level of most subjects, which inevitably leads to endogenous insulin secretion while glucose level adjustment before and during the clamp study. C-peptide and insulin measures are only available from 15 minutes before study drug administration, but glucose adjustment started 120 minutes before insulin administration. It is acknowledged that C-peptide measures during the study suggest suppression of endogenous insulin compared to pre-dose levels. However, the pre-dose C-peptide data indicate levels above levels expected for a fasted state and C-peptide does not appear fully suppressed after study insulin administration. In conclusion it remains unclear whether endogenous insulin secretion prior and during the study might have interfered with PK/PD measures, which questions the reliability of the comparison for biosimilarity of the products. Due to these concerns on the study design with clamps being performed at 6mmol/L, it cannot be excluded that the elicited endogenous insulin secretion interfered with the study measures and therefore biosimilarity of Insulin Human Rechon to Humulin is not considered unambiguously demonstrated yet. As this is essential for the conclusion on biosimilarity, the available data from study RCT-001 are presently not sufficient to establish PK/PD equivalence of insulin Rechon versus the reference. Compelling evidence would be required to mitigate the outlined concern (**MO**).

3.3.4. Clinical efficacy

No data on clinical efficacy were provided for this biosimilar application.

3.3.5. Discussion on clinical efficacy

No clinical efficacy studies were conducted, which is acceptable according to the respective GL (EMEA/CHMP/BMWP/32775/2005_Rev. 1), which states that "*there is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered sensitive enough to detect potentially clinically relevant differences between two insulins.*"

3.3.6. Clinical safety

Safety of Insulin Human Rechon has been assessed in healthy volunteers (phase 1 study (RCT-001)) and in type 1 Diabetic patients (phase 3 study (RCT-004)). Phase 1 Pharmacokinetic/Pharmacodynamic study (Study Code RCT-001): Safety and tolerability of Insulin Human Rechon were assessed in healthy volunteers in the phase 1 study (RCT- 001) after single subcutaneous doses of Insulin Human Rechon and Humulin® Regular. Phase 3 Safety study (Study Code RCT-004): Long-term safety of Insulin Human Rechon compared to Humulin® Regular in terms of immunogenicity, insulin tolerance and general safety variables, is assessed in a randomised phase 3 trial (RCT-004) in type 1 Diabetic patients. This study has been performed after positive nonclinical studies results, and a phase 1 study (RCT-001) conducted in healthy volunteers, where pharmacokinetic (PK), pharmacodynamic (PD) and safety parameters were assessed. For more details regarding study design and study population of study RCT-001, please refer to section 3.3.1.2.

Study RCT-004

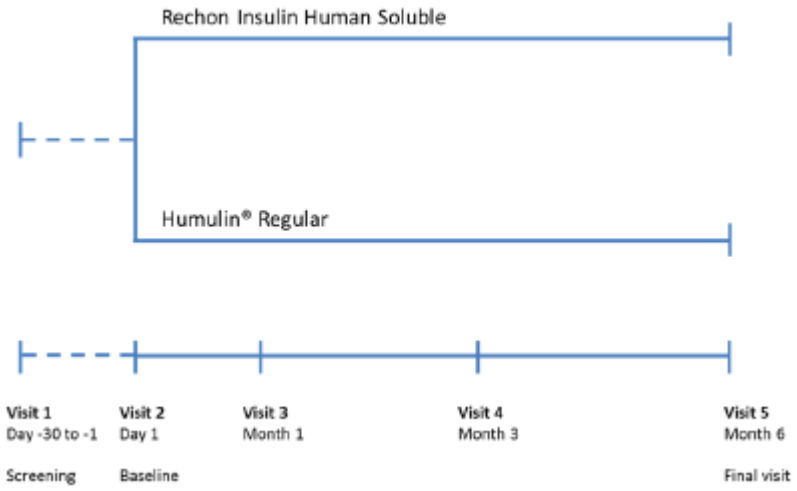
Overall Study Design and Plan of study RCT-004

The study was conducted as a multi-centre, randomised, open, parallel-group study to examine the long-term safety, focusing on immunogenicity, of Insulin Human Rechon as compared to Humulin® Regular. The patients were randomised 1:1 to treatment with Insulin Human Rechon or to treatment with Humulin® Regular.

The study included 5 visits; at Visit 1 (screening visit) patients were assessed for eligibility, at Visit 2 patients received IMP to be injected daily at home and at Visit 3 (month 1), Visit 4 (month 3) and Visit 5 (month 6) patients were asked to come to the study centre for safety assessments. The screening visit (Visit 1) took place no more than 1 month before the first dosing. The total study duration for each patient was approximately 7 months, including the screening visit.

This was a multicentre study performed in Germany and Poland.

Figure 12: Overall Study Design of Study RCT-004



Study Population

Inclusion Criteria

The patients had to meet all of the following criteria:

1) Willing and able to provide informed consent

2) Male or female diagnosed with T1DM for at least 2 years

3) Ongoing daily treatment with insulin for at least 12 months

4) Current treatment with insulin below or equal to 1.2 U/kg/day

5) Age 18-75 (both inclusive) at the time of signing informed consent

6) Body Mass Index (BMI) 18.0-32.0 kg/m2 (both inclusive)

7) HbA1c (glycosylated haemoglobin A1c) below or equal to 94 mmol/mol

8) Women had to be:

• postmenopausal, defined as:

above 45 years of age with amenorrhea for at least 18 months, or above 45 years of age with amenorrhea for at least 6 months and less than 18 months and a known serum follicle stimulating hormone (FSH) level above 40 IU/L, or

• surgically sterile (have had a hysterectomy or bilateral oophorectomy, tubal occlusion), or otherwise be incapable of pregnancy, or

• heterosexually active and practicing a highly effective method of birth control, including hormonal prescription oral contraceptives, contraceptive injections, contraceptive patch, intrauterine device, or male partner sterilization, consistent with local regulations regarding use of birth control methods for patients participating in clinical trials, for the duration of their participation in the study, or

• not heterosexually active. Note: patients who are not heterosexually active at screening must agree to utilize a highly effective method of birth control if they become heterosexually active during their participation in the study

9) Women of childbearing potential (i.e., those patients who did not meet the postmenopausal definition above, regardless of age) had to have a negative urine pregnancy test at baseline (Day 1) and at screening if required by local regulations (Note: a serum pregnancy test was acceptable in lieu of a urine pregnancy test, if required by local regulations)

Exclusion Criteria

The patients were not to meet any of the following criteria:

1) Known allergies, hypersensitivity, or intolerance to the IMP or its excipients

2) Renal disease that requires treatment with immunosuppressive therapy or a history of chronic dialysis or renal transplant. Note: patients with a history of treated childhood renal disease without sequelae may participate

3) Myocardial infarction, unstable angina, revascularisation procedure, or cerebrovascular accident within 3 months before screening, or a planned revascularisation procedure, or history of New York Heart Association (NYHA) Class IV cardiac disease (The Criteria Committee of the New York Heart Association)

4) Known history of hepatitis B surface antigen or hepatitis C antibody positive (unless known to be associated with documented persistently stable/normal range aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels), or other clinically active liver disease

5) Female patients: pregnant or breast-feeding or planned to become pregnant or breastfeed during the study

6) History of malignancy within 5 years before screening (exceptions: squamous and basal cell carcinomas of the skin and carcinoma of the cervix in situ, or a malignancy that in the opinion of the Investigator, were considered cured with minimal risk of recurrence)

7) History of human immunodeficiency virus (HIV) antibody positive

8) History of one or more severe hypoglycaemic episodes within 6 months before screening Note: a severe hypoglycaemic episode was defined as an event that required the help of another person. This referred to hypoglycaemic episodes, which were correlated to the patient's insulin sensitivity and not caused by incidents as for example medication error or acute in hospital treatment for other reasons (as clarified in CSP version 6.0).

9) Investigator's assessment that the patient's life expectancy was less than 1 year, or any condition, for example patients with severe co-diseases, that in the opinion of the Investigator would have made participation not in the best interest of the patient, would have interfered with the patients ability to participate in all study visits and complete the whole study, or could prevent, limit, or confound the protocol-specified safety assessments (as clarified in CSP version 6.0).

10)Major surgery (i.e. requiring general anaesthesia) within 3 months of the screening visit or any surgery planned during the patient's expected participation in the study (except minor surgery, i.e. outpatient surgery under local anaesthesia)

11) Any condition that, in the opinion of the Investigator, would have compromised the wellbeing of the patient or prevented the patient from meeting or performing study requirements

12) Current use of a corticosteroid medication or immunosuppressive agents, or likely to require treatment with a corticosteroid medication (for longer than 2 weeks in duration) or an immunosuppressive agent. Note: patients that used inhaled, intranasal, intra-articular, or topical corticosteroids, or corticosteroids in therapeutic replacement doses could participate

13) Use of other antidiabetic therapy than insulin

14) Received an active investigational drug (including vaccines) or used an investigational medical device within 3 months before Day 1/baseline

15) Employees of the Investigator or study centre, with direct involvement in the proposed study or other studies under the direction of that Investigator or study centre, as well as family members of the employees or the Investigator

16) More than 1 diabetic ketoacidosis requiring hospitalisation within the last 3 months prior to Visit 1

Restrictions

Women of childbearing potential who were heterosexually active were to practice a highly effective method of birth control, including hormonal prescription oral contraceptives, contraceptive injections, contraceptive patch, intrauterine device, or male partner sterilization during study participation, from signature of ICF until Visit 5.

Treatments study RCT-004

The term IMP includes Insulin Human Rechon and Humulin® Regular.

Patients received either Insulin Human Rechon or Humulin® Regular in a randomised way. The IMP was administered at home by the patients themselves. Dosing was individualised and adjusted based on blood glucose levels for each patient.

Before a meal, fast acting insulins (e.g. Humulin® Regular) are taken to be able to utilise the carbohydrates in the food and prevent blood glucose levels to rise. The more carbohydrates the food contains, the more insulin is needed.

Long-acting or intermediate-acting insulins are taken to slow the release of glucose from the liver during the periods when the patient does not eat, thereby keeping the blood glucose at a steady level. There are several different insulins on the market with different durations of action to maintain blood glucose levels at a steady level. The patients were to continue to take their long-acting or intermediate-acting insulins as usual. The type or dosing frequency of the long-acting or intermediate-acting insulins were not changed during the study.

The dosage of both long/intermediate-acting insulins and fast-acting insulins was always adjusted individually and determined by the response in blood glucose levels.

Identity of Investigational Medicinal Products

Information on the fast-acting insulin IMPs administered during the study is given in the table below.

Table 15: Investigational Medicinal Products

	Investigational Medicinal Product	
	Test Product	**Reference Product**
Product name	Insulin Human Rechon	Humulin® Regular
Source	*E. coli*	*E. coli*
Dosage form	Injection solution	Injection solution
Unit strength	100 IU/ml	100 IU/ml
Appearance	Clear colorless solution	Clear colorless solution
Excipients	Glycerol M-cresol Hydrochloric acid Sodium hydroxide Water for injection	Glycerol M-cresol Hydrochloric acid Sodium hydroxide Water for injection
Administration	Subcutaneous injection	Subcutaneous injection
Mode of action	Fast-acting	Fast-acting

Insulin Human Rechon was provided in cartridges to be used in the insulin pen and with needles. The pen is documented for use with Rechon insulin products and is CE marked.

Humulin® Regular was provided in 3 mL cartridges to be used in the insulin pen manufactured by Eli Lilly and Company. The pen was intended for use with the Humulin® products.

Selection of Dose and Timing of Dose

Dosing as well as timing of dosing was individualised and adjusted based on blood glucose levels for each patient.

Blinding

Patients and Investigators were not blinded to study treatment in this study. It was not possible to completely blind the study. The primary variable in the study was anti-insulin antibody.

Treatment Compliance

Patients documented their daily intake of IMP in the patient diary. Treatment compliance was monitored at each visit to the study centre by checking the amount of administered IMP according to the diary.

Study Objectives and Endpoints for Study RCT-004

Primary Objective

The primary objective was to assess the long-term safety of Insulin Human Rechon as compared to Humulin® Regular in terms of immunogenicity and insulin tolerance.

Secondary Objectives

The secondary objective was to assess the long-term safety of Insulin Human Rechon as compared to Humulin® Regular in terms of general safety variables.

Primary Endpoint

The primary endpoint was the proportion of patients having binding antibodies against human insulin. The anti-insulin binding antibodies was to be followed from Visit 2 (baseline) to Visit 5.

Safety Assessment in study RCT-004

Primary Safety Variable and Assessments

Detection of binding antibodies against insulin was performed using a three-tiered approach:

Tier 1) Screening assay for detection of binding antibodies and potential positive samples,

Tier 2) Confirmatory assay performed to rule out false positive samples, and

Tier 3) Titre assay to determine the titre of binding antibodies in the confirmed positive samples.

Two fasting blood samples were taken from each patient at all study visits (Visit 2 to Visit 5) for determination of binding antibodies against human insulin.

Secondary Safety Variables and Assessments

Neutralizing Capacity

As a part of the assessment of insulin tolerance, the neutralizing capacity of the anti-insulin antibodies was to be determined at Visit 2 (baseline), Visit 3, Visit 4 and Visit 5 using a cell based assay. The cut-off limits for a neutralizing anti-insulin response was to be predetermined from normal blood donors in the validation of the neutralizing assay.

One (1) fasting blood sample was taken from each patient for determination of neutralizing capacity of the anti-insulin antibodies., these analyses were not performed due to technical issues.

Insulin Dose

As a part of the assessment of insulin tolerance, the insulin dose was evaluated during the period from Visit 2 to Visit 5. The patients noted every dose administration, including the long-acting or intermediate-acting insulin, in the patient diary during one week after Visit 2 and during one week before Visit 3, Visit 4, and Visit 5. The recordings were used for the evaluation.

Glycosylated Haemoglobin A1c

As a part of the assessment of insulin tolerance, HbA1c was measured at Visit 2, Visit 3, Visit 4 and Visit 5. In addition, HbA1c was measured for eligibility purposes at Visit 1 (screening). The analysis of HbA1c to assess eligibility at Visit 1 was performed locally at the study centres. Analyses of HbA1c to assess insulin tolerance during the study period from Visit 2 until Visit 5 were performed.

Fasting Plasma Glucose

As a part of the assessment of insulin tolerance, fasting plasma glucose was measured at Visit 2, Visit 3, Visit 4 and Visit 5.

Hypoglycaemic Episodes

As a part of the assessment of insulin tolerance, hypoglycaemic episodes were evaluated during the period from Visit 2 to Visit 5. The recordings in the patient diary were used for the evaluation. Both the total number of episodes and the subclasses according to the American Diabetes Association (ADA) classification were evaluated.

Definition of hypoglycaemia according to ADA:

1) Severe hypoglycaemia

Severe hypoglycaemia is an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the return of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

2) Documented symptomatic hypoglycaemia

Documented symptomatic hypoglycaemia is an event during which typical symptoms of hypoglycaemia are accompanied by a measured plasma glucose concentration ≤70 mg/dL (≤3.9 mmol/L).

3) Asymptomatic hypoglycaemia

Asymptomatic hypoglycaemia is an event not accompanied by typical symptoms of hypoglycaemia but with a measured plasma glucose concentration ≤70 mg/dL (≤3.9 mmol/L).

4) Probable symptomatic hypoglycaemia

Probable symptomatic hypoglycaemia is an event during which symptoms typical of hypoglycaemia are not accompanied by a plasma glucose determination but was presumably caused by a plasma glucose concentration ≤70 mg/dL (≤3.9 mmol/L).

5) Relative hypoglycaemia

Relative hypoglycaemia is an event during which the person with diabetes reports any of the typical symptoms of hypoglycaemia with a measured plasma glucose concentration >70 mg/dL (>3.9 mmol/L) but approaching that level.

Circulating IgE Antibodies

Blood samples for analysing levels of total circulating immunoglobulin E (IgE) antibodies were taken at Visit 2, Visit 3, Visit 4 and Visit 5..

Local or Systemic Hypersensitivity

Incidents of local or systemic hypersensitivity were documented by the patient in the study diary.

Laboratory Safety Assessments

The haematology and clinical chemistry analyses during the study period, from Visit 2 until Visit 5, were performedat a laboratory, while the analyses at Visit 1 (screening) were performed locally at the study centres. The urinalysis (dip-stick) was performed locally at the study centres at all study visits. The laboratory safety parameters that were analysed are presented in the table below.

Table 16: Laboratory Safety Parameters

Category	Laboratory Parameter
Haematology	Haemoglobin, haematocrit, white blood cells, red blood cells, platelets
Clinical Chemistry	C-reactive protein, creatinine, total bilirubin, ALT, AST, gamma-glutamyltransferase, sodium, potassium, alkaline phosphatase
Urinalysis	Erythrocytes, leukocytes, protein, nitrite, glucose

ALT=alanine aminotransferase, AST=aspartate aminotransferase

The observed values were recorded and assessed as "normal" or "abnormal". Abnormal findings were assessed as "clinically significant" (CS) or "not clinically significant" (NCS).

Body Weight

Body weight was measured at all study visits throughout the study.

Vital Signs

The following vital signs were monitored:

• Supine systolic and diastolic blood pressure (mmHg), after 5 minutes lying down

• Supine pulse/heart rate (beats per minute), after 5 minutes lying down

The observed values were recorded and assessed as "normal", "abnormal, NCS" or "abnormal, CS".

Physical Examination

Standard physical examinations included assessments of the general condition, heart, lungs and abdomen.

Adverse Events

All AEs were recorded during the study period from first dose of IMP until the completion of Visit 5. The Investigator collected AEs at all study visits based on asking non-leading questions, making direct observations and by spontaneously volunteered information from patients. Clearly related signs, symptoms and abnormal diagnostic procedure results were to be grouped together and reported as a single diagnosis or syndrome whenever possible.

Detailed information on each AE was recorded in the eCRF, such as a brief description, start and stop date, severity, action taken regarding study drug, causality, seriousness and outcome. Any AE that was ongoing at the time when the patient left the study was to be followed up until the AE was resolved or the Investigator decided that the AE was stable and did not need further follow-up.

AEs of special interest (AESI) included:

• Injection site reactions

• Immunological reactions (e.g. drug hypersensitivity reactions involving anaphylaxis/anaphylactoid reactions, acute bronchoconstriction, angioedema, and/or acute urticaria)

• Severe hypoglycaemic events. Hypoglycaemia was only reported as an AE if it fulfilled a serious criteria. All other hypoglycaemic events were noted in the diary. Note: a severe hypoglycaemic event was defined as an event that required the help of another person.

• Events of hypokalaemia. Note: a hypokalaemia event is defined as the need for potassium substitution due to acute hypokalaemia by the discretion of the Investigator

Safety Assessment in study RCT-001

Safety and tolerability of Insulin Human Rechon were judged by:

- Adverse events
- Incidents of local intolerance
- Clinical chemistry (potassium)

The reporting period spanned the first dose of IMP until the completion of the follow-up visit (Visit 3). Incidents of local intolerance at the site of injection, if any, were planned to be reported as AEs. The following other safety assessments were performed during the study (see also "Study procedures" for further timings of safety assessments):

- Physical examinations (Standard physical examinations including assessments of the general condition, the ears, nose, throat, heart, lungs and abdomen)
- Vital signs (Supine systolic and diastolic blood pressure (mmHg) and pulse/ heart rate after 5 minutes lying down; oral body temperature)
- ECG
- Laboratory safety evaluation (haematology, clinical chemistry and urinalysis)

The timings of laboratory safety analyses are displayed below:

		Visit 1 Screening	Visit 2				Visit 3, Follow-up
			Dose 1		Dose 2		
			Before dose	0-10 h	Before dose	0-10 h	
Haematology	HbA1c	X					
	WBC	X					X
	RBC	X					X
	Haematocrit	X					X
	Haemoglobin	X	X		X		X
	Platelets	X					X
Clinical Chemistry	CRP	X	X		X		X
	Creatinine	X					X
	Total bilirubin	X					X
	ALT	X					X
	AST	X					X
	Sodium	X					X
	Potassium	X	X	X[a]	X	X[a]	X
	Glucose	X					X
Urinalysis	Erythrocytes	X					X
	Leukocytes	X					X
	Protein	X					X
	Glucose	X					X
	Nitrite	X					X

[a] Blood samples for potassium will be drawn at 2 h, 4 h and 8 h post dose

Statistical methods

For details regarding statistical methods of study RCT-001 please refer to section 3.3.1.2.. Below information refers to study RCT-004.

Data Sets to be Analysed

The patient population sets were defined as follows:

Safety set: All randomised patients who took at least 1 dose of the study drug.

Per protocol set (PPS): The PPS was defined for each study visit to consist of all randomised patients who completed the study up to the specific visit and were deemed to have no major protocol deviations that could interfere with the objectives of this study. The visit-specific PPS are sub-sets of the safety set.

Note: Important violations of eligibility criteria and other deviations from the protocol were assessed at a pre-analysis review meeting when all data was available, before database lock. It was planned in the CSP and SAP that important deviations from the protocol could lead to exclusion of a patient from the PPS, whereas exclusion of patients from the safety set was also considered at the pre-analysis review meeting. The final criteria for exclusion from the safety set and PPS are summarised in Section 11.1 and further detailed in the pre-analysis review report (Appendix 16.1.9).

Data was summarised and analysed using both the safety set and the PPS:

• For the primary endpoint, PPS was the primary analysis population.

• Baseline characteristics, AEs and concomitant medication were summarised for the safety set.

Patients were analysed in accordance with the treatment actually taken.

Definitions

For the primary endpoint baseline was defined as Visit 2 (Day 1). For all other endpoints, a baseline assessment was defined as the latest available non-missing assessment made before first administration of IMP, i.e. Visit 1 or Visit 2.

Date of first administration of IMP was determined using the patient diaries. If the information was missing, the date of the IMP was assumed to be the date of Visit 2.

Relative day was derived as:

> Relative day = (Start date) - (Date of first administration of IMP) + 1.
>
> For events occurring or starting before the date of first administration of IMP, the relative day was derived as:
>
> Relative day = (Start date) - (Date of first administration of IMP)
>
> In this way, there was no Day 0. Day 1 is the same day as the day of first administration of IMP, and Day -1 is the day before.

Statistical issues

As the main objective of conducting the study was safety, statistical testing of hypotheses would only be considered exploratory. Lack of significance would not be evidence of similarity. All statistical models applied were foremost used as tools for estimating treatment differences rather than testing hypotheses. Furthermore, it should be noted that any model estimated CIs or p-values indicating statistically significant difference between treatments should be interpreted as suggestive rather than evidence of differences, since corrections for multiple testing were not applied in this study.

Summary statistics

In general, data was summarised by means of summary statistics. Continuous data was presented with the number of observations, mean value, standard deviation, minimum, Q1, median, Q3 and maximum value. Categorical data was presented as counts and percentages. The denominator for percentages was the number of patients with non-missing data, unless otherwise specified. The data was presented for each treatment group, parameter category, parameter and by visit, as applicable.

Primary safety analysis

The number and percentages of patients having positive anti-insulin binding antibodies were tabulated for each visit and a corresponding shift from baseline table was presented. Ninetyfive (95) percent CIs (based on normal approximation) for the percentage in each treatment group as well as for the difference between the 2 treatment groups was given (calculated as described in literature reference 13). Ninety-five (95) percent CIs for the percentages in shift tables was given for each treatment group as well as for the difference between the treatment groups.

Sensitivity analyses were performed to assess the impact of missing data. Since this was a study comparing 2 biosimilar products, missing at random could be justified and the difference between the 2 groups was to be evaluated using both unweighted and weighted generalised estimating equations.

Secondary safety analyses

Mostly summary statistics were produced to depict observed values and for changes from baseline for secondary endpoints (see above).

Sample size

No formal sample size calculation was done. It was judged that 150 randomised patients in each treatment group will be enough to meet the primary objective of the study. The study was not powered to formally demonstrate non-inferiority regarding immunogenicity.

With 150 patients in each group, the maximum width of a CI for the difference in the change in the percentage of patients having a B/T% above the cut-off limit will be ±11%.

To avoid a decrease in sample size due to dropout, the number of dropouts will be monitored during the study and the sample size will be adjusted accordingly. The maximum number of randomised patients will be 330.

Study conduct

For details regarding study conduct of study RCT-001 please refer to section 3.3.1.2.. Below information refers to study RCT-004.

Changes in the Conduct of the Study or Planned Analyses

Substantial Amendment 05 December 2017

Prior to this amendment, there were two local versions of the CSP; version 3.0 that was approved only in Poland and version 4.0 that was approved only in Germany. This substantial amendment was made to combine information in the two local CSP versions into a single global version of the CSP. This substantial amendment included: addition of a withdrawal criterion regarding pregnancy, updated information on origin of source data and medical records, correction to the list of references. This substantial amendment rendered the global CSP version 5.0.

Substantial Amendment 25 June 2018

The analysis method for the primary variable was changed to a 3-tiered approach with a new method of analysis and procedure for the detection of antibodies against insulin. A clarification was made for the re-check of eligibility criteria, where laboratory safety assessments were to be performed. Exclusion criteria regarding definition of hypoglycaemic episodes and patient life expectancy were updated.

Definition and reporting procedure for suspected unexpected serious adverse reactions (SUSARs), as well as clarification of follow-up of patients with ongoing AEs at database lock was added. In addition, clarification of when hypoglycaemic events should be reported as an AE was made.

A sample size adjustment was added to avoid a decrease in sample size due to dropout and the planned study start and end dates were updated. Information regarding data protection was updated in light of new GDPR legislation. Minor administrative and editorial changes were made.

Substantial Amendment 01 rendered the CSP version 6.0.

Changes in the Planned Analyses

Samples were collected for assessment of neutralizing capacity of anti-insulin antibodies, however the samples were not analysed prior to the database closure and results are therefore not included in this clinical study report. Samples were not analysed due to technical challenges of establishing a validated assay method for assessing the neutralizing capacity of the anti-insulin antibodies. This analysis was however only included in the CSP based on a recommendation made by the CHMP to perform if possible, not as a requirement.

Although it was planned to evaluate exclusions only from the PPS at the pre-analysis review meeting, patients were also assessed for exclusion from the safety set. The excluded patients and the reasons for exclusion are detailed in the preanalysis review report.

For the primary safety variable, unweighted generalised estimating equations were not used to evaluate the difference between the groups in presence of anti-insulin antibodies. Only observation-specific weighted generalised estimating equations were used.

3.3.6.1. Analytical methods

Binding antibodies

Detection of binding antibodies against insulin was performed using a three-tiered approach:

Tier 1) Screening assay for detection of binding antibodies and potential positive samples,

Tier 2) Confirmatory assay performed to rule out false positive samples, and

Tier 3) Titre assay to determine the titre of binding antibodies in the confirmed positive samples.

Two fasting blood samples were taken from each patient at all study visits (Visit 2 to Visit 5) for determination of binding antibodies against human insulin.

Neutralizing antibodies

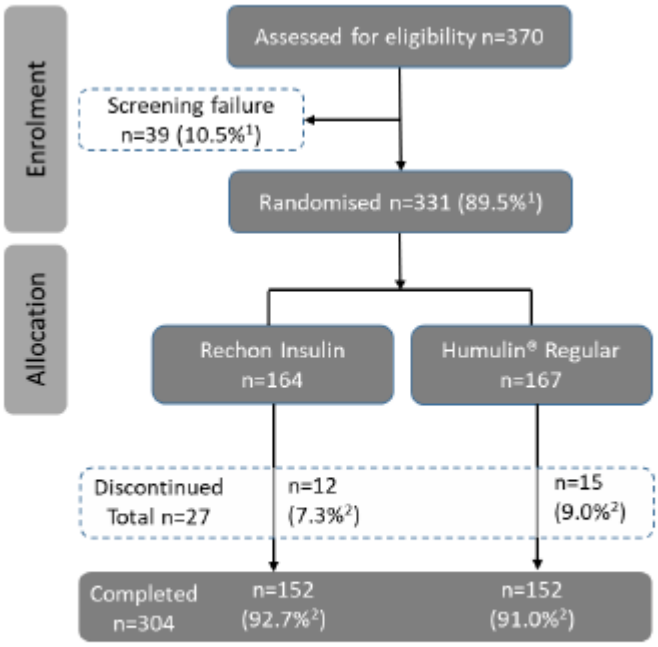
Determination of neutralizing capacity of the anti-insulin antibodies was not performed due to technical challenges of establishing a validated assay method.

3.3.6.2. Patient exposure

For details regarding patient exposure of study RCT-001 please refer to section 3.3.1.2. Below information refers to study RCT-004.

In study RCT-004, 370 patients were screened for participation, and as 39 patients (10.5%) were screening failures, 331 patients (89.5%) were randomized to treatment with either Rechon Insulin (164 patients) or Humulin Regular (167 patients). In total, 8.2% of the randomised patients (n=27) discontinued the study; 7.3% of the patients (n=12) randomised to treatment with Rechon Insulin and 9.0% of the patients (n=15) randomised to treatment with Humulin Regular.

Figure 13: Patient Disposition



Source: Table 14.1.1
[1]Number of patients assessed for eligibility is denominator for percentage calculations
[2]Number of patients allocated in subgroup is denominator for percentage calculations

Table 17: Reasons for Discontinuation (All Randomised)

	Rechon Insulin n=164	Humulin® Regular n=167	Total n=331
Discontinued patients	12 (7.3%)	15 (9.0%)	27 (8.2%)
Withdrawal of consent	8	9	17
Adverse Event	2	3	5
Lost to follow-up	0	1	1
Protocol deviation	0	1	1
Other	2	1	3

Source: Table 14.1.1
n = number of randomised patients, used as denominator for percentage calculations

Protocol Deviations

.

Out of the all major protocol deviations, only 2 protocol deviations were assessed to have an impact on data analysis. Consequently, parts of the data from patient A (Insulin Human Rechon, prohibited medication) and patient B (Humulin Regular, patient compliance with treatment) were excluded from the PPS as detailed in the table below.

In addition, there were a number of minor protocol deviations recorded during the study.

Data Sets Analysed

In total, 0.9% of the patients (n=3), all randomised to treatment with Humulin Regular, were excluded from the safety set. As detailed in the pre-analysis review report, 2 out of these 3 patients were excluded due to too short study participation, which made it unclear if they ever took any study

medication, and 1 patient was excluded due to erroneous randomisation despite being hepatitis B (HBsAg) positive (exclusion criterion 4).

An additional 3.3% of the patients (n=11) had at least some data that was excluded from the PPS on a per-visit basis.

Table 18: Exclusion from safety Set (All Randomised)

	Rechon Insulin n=164	Humulin® Regular n=167	Total n=331
Excluded from Safety Set	**0 (0.0%)**	**3 (1.8%)**	**3 (0.9%)**
Reason for exclusion			
Erroneously randomised	0	1	1
Too short study participation	0	2	2
Excluded from PPS[1]	**8**	**3**	**11**

Source: Table 14.1.2, Table 14.1.3
[1]Exclusion from PPS is on a per-visit basis, as detailed in Table 8. PPS is a subset of the safety set, but only exclusions made in addition to those patients already excluded from the safety set are presented.
PPS = per protocol set

Table 19: Data Excluded from the Per Protocol Set

Reason for exclusion from PPS	Rechon Insulin n=164	Humulin® Regular n=164
Diary data for diary 2 excluded due to large deviations in diary date in relation visit date.	3	0
Diary data for diary 3 excluded due to large deviations in diary date in relation visit date.	1	0
Anti-insulin antibodies data for visit 3 and data from diary 1 (visits 2 and 3) excluded due to wrong insulin taken.	1[1]	0
All anti-insulin antibodies data and all diary data excluded due to interruption of trial medication and patient withdrawn for safety concerns.	1	0
Anti-insulin antibodies data for visit 5 and data from diary 3 (Visit 5) excluded due to wrong insulin dispensed by mistake.	1	0
Anti-insulin antibodies data for visit 5 and all diary data excluded due to wrong insulin taken.	1	0
Anti-insulin antibodies data diary data for visit 5 (diary 3) excluded due to wrong insulin taken.	0	1[2]
Anti-insulin antibodies data for visit 3 excluded due to withdrawal of informed consent	0	1
All anti-insulin antibodies data and all diary data excluded due to wrong insulin taken.	0	1

Source: Table 14.1.2, Table 14.1.3
Data excluded due to major protocol deviations as described in Section 10.2: [1]Patient A1307, [2]Patient B1343

Demographic and other baseline characteristics

For details regarding demographic and baseline characteristics of study RCT-001 please refer to section 3.3.1.2. Below information refers to study RCT-004.

<u>Demography</u>

Table 20: Demography (Safety Set)

		Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
Age (years)	Mean (SD)	46.2 (12.8)	45.5 (13.2)	45.9 (13.0)
	Median	46.0	45.0	46.0
	Q1, Q3	37.0, 56.5	36.0, 55.0	36.0, 56.0
	Min, Max	18, 74	18, 73	18, 74
Sex n (%)	Female	86 (52.4%)	65 (39.6%)	151 (46.0%)
	Male	78 (47.6%)	99 (60.4%)	177 (54.0%)
Race n (%)	White	163 (99.4%)	164 (100%)	327 (99.7%)
	White, Other	1 (0.6%)	0 (0.0%)	1 (0.3%)
Country	Germany	5 (3.0%)	3 (1.8%)	8 (2.4%)
	Poland	159 (97.0%)	161 (98.2%)	320 (97.6%)

Source: Table 14.1.4
Q1/Q3 = 1st/3rd quartile; SD = standard deviation

The demographic characteristics in the PPS were similar to those in the safety set, where the overall mean age in the PPS was 45.9 years and 47.0% of the patients (n=149) were female. The slightly skewed gender distribution noticed in the Humulin Regular group in the safety set was also apparent in the PPS, where 40.0% of the patients (n=64) were female. Similar to the safety set, 99.7% of the patients (n=316) in the PPS were White and 97.5% of the patients (n=309) were recruited in Poland. All other patients were recruited in Germany.

Medical history

RCT-001

Only one subject had any medical history of interest (past histories of tonsillectomy and inguinal hernia operation).

RCT-004

Table 21: Medical History Reported by >5% of Patients in Either Treatment Group (Safety Set)

Dictionary derived term	Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
Hypertension	69 (42.1%)	68 (41.5%)	137 (41.8%)
Diabetic retinopathy	39 (23.8%)	27 (16.5%)	66 (20.1%)
Diabetic neuropathy	31 (18.9%)	24 (14.6%)	55 (16.8%)
Hypothyroidism	22 (13.4%)	12 (7.3%)	34 (10.4%)
Hypercholesterolaemia	15 (9.1%)	19 (11.6%)	34 (10.4%)
Hyperlipidaemia	18 (11.0%)	15 (9.1%)	33 (10.1%)
Autoimmune thyroiditis	14 (8.5%)	14 (8.5%)	28 (8.5%)
Autonomic neuropathy	11 (6.7%)	11 (6.7%)	22 (6.7%)
Dyslipidaemia	7 (4.3%)	12 (7.3%)	19 (5.8%)
Diabetic nephropathy	10 (6.1%)	8 (4.9%)	18 (5.5%)
Lipid metabolism disorder	7 (4.3%)	10 (6.1%)	17 (5.2%)
Appendectomy	6 (3.7%)	10 (6.1%)	16 (4.9%)
Asthma	9 (5.5%)	7 (4.3%)	16 (4.9%)
Cataract	9 (5.5%)	7 (4.3%)	16 (4.9%)

Source: Table 14.1.7
n = used as denominator for percentage calculation

Prior and concomitant medication

Study RCT-001

Concomitant medication taken during the study were provided as a list for each subject and summarized as table. Per protocol, use of any medicinal product, prescription or over-the-counter was prohibited within 2 weeks prior to the administration of IMP and throughout the study, except if this would not affect the outcome of the study as judged by the Investigator. In addition, the subjects were not allowed to drink anything but water from the evening before the euglycaemic clamp study and until the end of the clamp. However, a decision was taken during the study to allow one cup of black coffee before start of the euglycaemic clamp (at the discretion of the Investigator), if the subject was at risk of developing headache without coffee. This was documented in the Declaration of Database Lock document. Caffeine (a cup of coffee) was taken by a total of 4 subjects during dose days. Two subjects had coffee on both Day 1 and Day 2, whereas 2 subjects had coffee on only one of these days. In addition, 3 subjects were treated with potassium citrate (2 subjects after administration of Insulin Human Rechon and 1 subject after administration of Humulin® Regular) due to low blood potassium. One additional subject took potassium citrate after administration of Insulin Human Rechon due to low levels of potassium (not clinically significant). Other AEs treated with concomitant medication were headache in Subjects (after administration of both Insulin Human Rechon and Humulin® Regular) and (after administration of Humulin® Regular), which were treated with paracetamol.

Study RCT-004

As expected from the patient population, the most common concomitant medication was insulin and analogues, where insulin glargine was used by 65.5% of the patients in total (n=215), with a similar distribution between the treatment groups; 62.8% of the patients (n=103) in the Insulin Human Rechon group and 68.3% of the patients (n=112) in the Humulin Regular group. Other common insulin and analogues used concomitantly were insulin human injection isophane, used by 22.0% of the patients in total (n=72) and insulin lispro used by 12.2% of the patients in total (n=40), both with a similar distribution between treatment groups. Other common concomitant medications, used by >10% of the patients, were thyroid preparations, ACE inhibitors, lipid-modifying agents and antithrombotic agents.

Table 22: Concomitant Medication Used by >10% of the Patients (Safety Set)

ATC3 term Preferred name	Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
Insulin and analogues			
Insulin glargine	103 (62.8%)	112 (68.3%)	215 (65.5%)
Insulin human injection, isophane	37 (22.6%)	35 (21.3%)	72 (22.0%)
Insulin lispro	21 (12.8%)	19 (11.6%)	40 (12.2%)
Insulin detemir	23 (14.0%)	13 (7.9%)	36 (11.0%)
Insulin human	18 (11.0%)	15 (9.1%)	33 (10.1%)
Thyroid preparations			
Levothyroxine sodium	31 (18.9%)	23 (14.0%)	54 (16.5%)
Ace inhibitors			
Ramipril	27 (16.5%)	26 (15.9%)	53 (16.2%)
Lipid-modifying agents			
Atorvastatin calcium	18 (11.0%)	20 (12.2%)	38 (11.6%)
Antithrombotic agents			
Acetylsalicylic acid	17 (10.4%)	17 (10.4%)	34 (10.4%)

Source: Table 14.3.10
ATC3 = anatomical therapeutic chemical (World Health Organization Drug Dictionary) level 3; n = used as denominator for percentage calculation

Treatment compliance

Table 23: Patient Compliance per Visit (Safety Set)

Number of Patients by Visit n (%)	Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
Visit 1 (Screening)	164 (100%)	164 (100%)	328 (100%)
Visit 2 (Day 1)	164 (100%)	164 (100%)	328 (100%)
Visit 3 (Day 30)	162 (98.8%)	160 (97.6%)	322 (98.2%)
Visit 4 (Day 90)	155 (94.5%)	155 (94.5%)	310 (94.5%)
Visit 5 (Day 180)	152 (92.7%)	152 (92.7%)	304 (92.7%)

Source: Table 14.1.6
n = used as denominator for percentage calculation

Total dose

Total daily insulin

Table 24: Total Daily Insulin Dose by Type of Insulin at Baseline (Safety Set)

Total dose (IU/kg), Absolute value		Intermediate-acting insulin		Long/ultralong-acting insulin	
		Rechon Insulin n=164	Humulin® Regular n=164	Rechon Insulin n=164	Humulin® Regular n=164
Visit 2 Derived value	n[a]	14	10	131	142
	Mean (SD)	0.703 (0.191)	0.656 (0.190)	0.682 (0.212)	0.679 (0.202)
	Median	0.652	0.606	0.641	0.665
	Q1, Q3	0.572, 0.929	0.513, 0.846	0.526, 0.825	0.533, 0.799
	Min, Max	0.37, 1.03	0.39, 0.94	0.29, 1.23	0.25, 1.18
Visit 5 Change from baseline	n[a]	12	10	119	126
	Mean (SD)	0.038 (0.094)	0.007 (0.092)	0.031 (0.115)	0.038 (0.111)
	Median	0.027	0.029	0.023	0.027
	Q1, Q3	-0.029, 0.089	-0.061, 0.087	-0.024, 0.097	-0.024, 0.095
	Min, Max	-0.09, 0.20	-0.15, 0.11	-0.44, 0.34	-0.33, 0.47

Source: Table 14.3.5.3
n[a] = patients with non-missing data
Q1/Q3 = 1st/3rd quartile; SD = standard deviation

Total bolus insulin

Table 25: Daily Bolus Insulin Dose by Type of Insulin at Baseline (Safety Set)

Bolus dose (IU/kg), Absolute value		Intermediate-acting insulin		Long/ultralong-acting insulin	
		Rechon Insulin n=164	Humulin® Regular n=164	Rechon Insulin n=164	Humulin® Regular n=164
Visit 2 Derived value	n[a]	14	10	131	142
	Mean (SD)	0.446 (0.153)	0.422 (0.142)	0.420 (0.161)	0.413 (0.156)
	Median	0.432	0.422	0.391	0.383
	Q1, Q3	0.339, 0.571	0.324, 0.457	0.296, 0.500	0.302, 0.490
	Min, Max	0.20, 0.70	0.18, 0.66	0.14, 0.97	0.11, 0.98
Visit 5 Change from baseline	n[a]	12	10	119	126
	Mean (SD)	0.042 (0.098)	0.006 (0.075)	0.034 (0.096)	0.034 (0.105)
	Median	0.031	0.019	0.032	0.025
	Q1, Q3	-0.025, 0.078	-0.038, 0.040	-0.022, 0.090	-0.020, 0.081
	Min, Max	-0.09, 0.23	-0.11, 0.15	-0.20, 0.33	-0.25, 0.46

Source: Table 14.3.5.3
n[a] = patients with non-missing data
Q1/Q3 = 1st/3rd quartile; SD = standard deviation

3.3.6.3. Adverse events

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Table 26: Adverse Events by System Organ Class and Preferred Term (Safety Set)

System Organ Class Preferred Term [a]		Rechon Insulin Human Soluble (n=22)	Humulin Regular (n=22)
Number of unique AEs		n = 8	n = 9
Number of subjects with at least one related AE		n = 3	n = 1
Number of subjects with at least one AE		n = 8	n = 7
General disorders and administration site conditions	Unique AEs	3	1
	Subjects	3 (13.6 %)	1 (4.5 %)
Fatigue		1 (4.5 %) 1	0
Catheter site pain		2 (9.1 %) 2	1 (4.5 %) 1
Investigations	Unique AEs	2	1
	Related	2	1
	Subjects	2 (9.1 %)	1 (4.5 %)
Blood potassium decreased		2 (9.1 %) 2	1 (4.5 %) 1
Nervous system disorders	Unique AEs	2	6
	Subjects	2 (9.1 %)	5 (22.7 %)
Dizziness		0	1 (4.5 %) 1
Hypoaesthesia		0	1 (4.5 %) 1
Headache		2 (9.1 %) 2	4 (18.2 %) 4
Metabolism and nutrition disorders	Unique AEs	1	0
	Related	1	0
	Subjects	1 (4.5 %)	0
Hypoglycaemia		1 (4.5 %) 1	0
Gastrointestinal disorders	Unique AEs	0	1
	Subjects	0	1 (4.5 %)
Vomiting		0	1 (4.5 %) 1

[a] MedDRA text from version 15.1
Unique AE = AE of a certain preferred term, counted only once within each subject and treatment
Related = Number of unique related AEs in system organ class
Subjects = Number of subjects with at least one AE in system organ class
x (xx.x%) y = number of subjects (percent of subjects with at least one AE) actual number of AEs
An AE is counted as related if relationship to study treatment is classified as possible or probable
Source: Appendix 16.2.7

RCT-004

AEs were recorded from first dose of IMP to the completion of Visit 5. As no AEs were collected before start of treatment, all reported AEs were treatment emergent. Hypoglycaemic episodes were only to be reported as AEs if they were considered severe, i.e. according to ADA classification (ADA category 1). All other cases of hypoglycaemia, ADA categories 2-5, were noted in the diary and not reported as AEs.

Table 27: Summary of Adverse Events (Safety Set)

Treatment group	Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
Adverse Events			
Number of AEs (unique AEs[1])	81 (79)	75 (72)	156 (151)
Number of patients with at least one event n (%)	46 (28.0%)	43 (26.2%)	89 (27.1%)
Serious Adverse Events			
Number of SAEs (unique SAEs[1])	10 (10)	9 (9)	19 (19)
Number of patients with at least one SAE n (%)	9 (5.5%)	9 (5.5%)	18 (5.5%)
Related Adverse Events			
Number of events (unique events[1])	17 (17)	7 (7)	24 (24)
Number of patients with at least one event n (%)	11 (6.7%)	7 (4.3%)	18 (5.5%)
Adverse Events Leading to Treatment Discontinuation			
Number of events (unique events[1])	10 (10)	5 (5)	15 (15)
Number of patients with at least one event n (%)	4 (2.4%)	5 (3.0%)	9 (2.7%)
Adverse Events Leading to Study Withdrawal			
Number of events (unique events[1])	2 (2)	3 (3)	5 (5)
Number of patients with at least one event n (%)	2 (1.2%)	3 (1.8%)	5 (1.5%)
Adverse Events Leading to Death			
Number of events (unique events[1])	0 (0)	2 (2)	2 (2)
Number of patients with at least one event n (%)	0 (0.0%)	2 (1.2%)	2 (0.6%)

Source: Table 14.3.1.1
[1]event of a certain preferred term, counted only once within each patient
n = used as denominator for percentage calculation

Table 28: Adverse Events Reported by ≥2 Patients in Either Treatment Group (Safety Set)

	Rechon Insulin n=164		Humulin® Regular n=164		Total n=328	
System Organ Class Preferred term[1]	Number of events	Number of patients with event	Number of events	Number of patients with event	Number of events	Number of patients with event
Infections and infestations	**21**	**18 (11.0%)**	**16**	**14 (8.5%)**	**37**	**32 (9.8%)**
Bronchitis	2	2 (1.2%)	1	1 (0.6%)	3	3 (0.9%)
Nasopharyngitis	2	2 (1.2%)	2	2 (1.2%)	4	4 (1.2%)
Rhinitis	2	2 (1.2%)	0	0 (0.0%)	2	2 (0.6%)
Sinusitis	2	2 (1.2%)	0	0 (0.0%)	2	2 (0.6%)
Urinary tract infection	4	4 (2.4%)	2	2 (1.2%)	6	6 (1.8%)
Metabolism and nutrition disorders	**9**	**9 (5.5%)**	**6**	**6 (3.7%)**	**15**	**15 (4.6%)**
Hyperglycaemia	3	3 (1.8%)	0	0 (0.0%)	3	3 (0.9%)
Hypoglycaemia	5	5 (3.0%)	3	3 (1.8%)	8	8 (2.4%)
Nervous system disorders	**6**	**6 (3.7%)**	**4**	**4 (2.4%)**	**10**	**10 (3.0%)**
Headache	3	3 (1.8%)	1	1 (0.6%)	4	4 (1.2%)
General disorders and administration site cond.[2]	**8**	**6 (3.7%)**	**4**	**3 (1.8%)**	**12**	**9 (2.7%)**
Injection site rash	2	2 (1.2%)	0	0 (0.0%)	2	2 (0.6%)
Peripheral swelling	2	2 (1.2%)	0	0 (0.0%)	2	2 (0.6%)
Gastrointestinal disorders	**5**	**4 (2.4%)**	**6**	**5 (3.0%)**	**11**	**9 (2.7%)**
Abdominal pain upper	0	0 (0.0%)	2	2 (1.2%)	2	2 (0.6%)
Nausea	2	2 (1.2%)	1	1 (0.6%)	3	3 (0.9%)
Musculoskeletal and connective tissue disorders	**7**	**6 (3.7%)**	**2**	**2 (1.2%)**	**9**	**8 (2.4%)**
Arthralgia	3	3 (1.8%)	0	0 (0.0%)	3	3 (0.9%)

Source: Table 14.3.1.2
[1]as defined by MedDRA version 20.1; [2]full name of SOC: General disorders and administration site conditions.
n = used as denominator for percentage calculation

The majority of all AEs were resolved or resolving at the end of the study. In the Insulin Human Rechon group, 7 patients had 1 AE each that was not resolved at the end of the study. All of these AEs were either of mild or moderate severity, where 1 AE led to discontinuation of study treatment (sinusitis,) and only 1 AE was assessed to be related to treatment (hyperglycaemia,).

In the Humulin Regular group, there were 6 patients that had in total 12 AEs that were not resolved at the end of the study. All of these AEs were either of mild or moderate severity, none of the AEs led to discontinuation of study treatment and only 1 AE was assessed to be related to treatment (increased blood alkaline phosphatase).

Related AEs

Table 29: treatment-Emergent Adverse events by System Organ Class and Preferred Term by Severity and Causality (Safety Set) – Related Adverse Events

	Rechon Insulin (n=164)		Humulin® Regular (n=164)		Total (n=328)	
	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]
METABOLISM AND NUTRITION DISORDERS	6	6 (3.7%)	4	4 (2.4%)	10	10 (3.0%)
Diabetes mellitus			1	1 (0.6%)	1	1 (0.3%)
Hyperglycaemia	3	3 (1.8%)			3	3 (0.9%)
Hypoglycaemia	3	3 (1.8%)	3	3 (1.8%)	6	6 (1.8%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	6	4 (2.4%)	1	1 (0.6%)	7	5 (1.5%)
Injection site erythema	1	1 (0.6%)			1	1 (0.3%)
Injection site pruritus	1	1 (0.6%)	1	1 (0.6%)	2	2 (0.6%)
Injection site rash	2	2 (1.2%)			2	2 (0.6%)
Peripheral swelling	2	2 (1.2%)			2	2 (0.6%)
INVESTIGATIONS	1	1 (0.6%)	2	2 (1.2%)	3	3 (0.9%)
Blood alkaline phosphatase increased			1	1 (0.6%)	1	1 (0.3%)
Blood glucose increased	1	1 (0.6%)	1	1 (0.6%)	2	2 (0.6%)
EYE DISORDERS	1	1 (0.6%)			1	1 (0.3%)
Lacrimation increased	1	1 (0.6%)			1	1 (0.3%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	2	1 (0.6%)			2	1 (0.3%)
Arthralgia	1	1 (0.6%)			1	1 (0.3%)
Muscle tightness	1	1 (0.6%)			1	1 (0.3%)
NERVOUS SYSTEM DISORDERS	1	1 (0.6%)			1	1 (0.3%)
Hypoaesthesia	1	1 (0.6%)			1	1 (0.3%)

[a] Unique within subject and preferred term.
[b] Number of subjects with at least one AE.
Source: List 16.2.7.1

Severe AEs

Table 30: treatment-Emergent Adverse events by System Organ Class and Preferred Term by Severity and Causality (Safety Set) – Severe Adverse Events

	Rechon Insulin (n=164)		Humulin® Regular (n=164)		Total (n=328)	
	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]
METABOLISM AND NUTRITION DISORDERS	5	5 (3.0%)	2	2 (1.2%)	7	7 (2.1%)
Hypoglycaemia	5	5 (3.0%)	2	2 (1.2%)	7	7 (2.1%)
GASTROINTESTINAL DISORDERS			1	1 (0.6%)	1	1 (0.3%)
Gastric haemorrhage			1	1 (0.6%)	1	1 (0.3%)
INFECTIONS AND INFESTATIONS	1	1 (0.6%)			1	1 (0.3%)
Appendicitis	1	1 (0.6%)			1	1 (0.3%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			1	1 (0.6%)	1	1 (0.3%)
Upper limb fracture			1	1 (0.6%)	1	1 (0.3%)
PSYCHIATRIC DISORDERS			1	1 (0.6%)	1	1 (0.3%)
Completed suicide			1	1 (0.6%)	1	1 (0.3%)
SURGICAL AND MEDICAL PROCEDURES			1	1 (0.6%)	1	1 (0.3%)
Fracture treatment			1	1 (0.6%)	1	1 (0.3%)

[a] Unique within subject and preferred term.
[b] Number of subjects with at least one AE.
Source: List 16.2.7.1

3.3.6.4. Serious adverse events, deaths, and other significant events

Deaths

There were 2 patients (1.2%) in the Humulin Regular group who died in the study due to suicide and gastric haemorrhage, respectively. Neither of the deaths was judged as related to the treatment. No patients died in the Insulin Human Rechon group.

Other serious adverse events

Including the 2 deaths, there were in total 10 SAEs reported by 5.5% of the patients (n=9) in the Insulin Human Rechon group and 9 SAEs reported by 5.5% of the patients (n=9) in the Humulin Regular group. Out of these, 3 SAEs were related to treatment with Insulin Human Rechon and 3 SAEs were related to treatment with Humulin Regular. All of the SAEs that were related to treatment were due to episodes of hypoglycaemia and classified as AESIs.

Treatment was discontinued due to SAE for 1 patient in the Insulin Human Rechon group (ketoacidosis, causality: unlikely) and for the 2 patients in the Humulin Regular group who died (causality: unlikely in both cases).

The patient in the Insulin Human Rechon group and the 2 patients in the Humulin Regular group who died, had SAEs that led to withdrawal from study participation. No patient was discontinued from treatment or withdrawn from the study due to a related SAE.

Table 31: Summary of Serious Adverse Events (Safety Set)

Treatment group	Rechon Insulin n=164	Humulin® Regular n=164	Total n=328
SAEs			
Number of SAEs (unique SAEs[1])	10 (10)	9 (9)	19 (19)
Number of patients with at least one event n (%)	9 (5.5%)	9 (5.5%)	18 (5.5%)
Related SAEs			
Number of events (unique events[1])	3 (3)	3 (3)	6 (6)
Number of patients with at least one event n (%)	3 (1.8%)	3 (1.8%)	6 (1.8%)
SAEs Leading to Treatment Discontinuation			
Number of events (unique events[1])	1 (1)	2 (2)	3 (3)
Number of patients with at least one event n (%)	1 (0.6%)	2 (1.2%)	3 (0.9%)
SAEs Leading to Study Withdrawal			
Number of events (unique events[1])	1 (1)	2 (2)	3 (3)
Number of patients with at least one event n (%)	1 (0.6%)	2 (1.2%)	3 (0.9%)

Source: Table 14.3.1.4
[1]event of a certain preferred term, counted only once within each patient
n = used as denominator for percentage calculation

Table 32: Serious Adverse Events (Safety Set)

	Rechon Insulin n=164		Humulin® Regular n=164		Total n=328	
System Organ Class Preferred term[1]	Number of events	Number of patients with event	Number of events	Number of patients with event	Number of events	Number of patients with event
Metabolism and nutrition disorders	6	6 (3.7%)	3	3 (1.8%)	9	9 (2.7%)
Hypoglycaemia	5	5 (3.0%)	3	3 (1.8%)	8	8 (2.4%)
Ketoacidosis	1	1 (0.6%)	0	0 (0.0%)	1	1 (0.3%)
Infections and infestations	3	3 (1.8%)	1	1 (0.6%)	4	4 (1.2%)
Appendicitis	1	1 (0.6%)	0	0 (0.0%)	1	1 (0.3%)
Diabetic foot infection	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Gastroenteritis	1	1 (0.6%)	0	0 (0.0%)	1	1 (0.3%)
Pneumonia	1	1 (0.6%)	0	0 (0.0%)	1	1 (0.3%)
Injury, poisoning and procedural complications	1	1 (0.6%)	1	1 (0.6%)	2	2 (0.6%)
Muscle rupture	1	1 (0.6%)	0	0 (0.0%)	1	1 (0.3%)
Upper limb fracture	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Gastrointestinal disorders	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Gastric haemorrhage	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Psychiatric disorders	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Completed suicide	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Cardiac disorders	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Bradycardia	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Vascular disorders	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Iliac artery occlusion	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)

Source: Table 14.3.1.5

Table 33: treatment-Emergent Adverse events by System Organ Class and Preferred Term by Severity and Causality (Safety Set) – Related Serious Adverse Events

	Rechon Insulin (n=164)		Humulin® Regular (n=164)		Total (n=328)	
	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]	Adverse Events [a]	Patients [b]
METABOLISM AND NUTRITION DISORDERS	3	3 (1.8%)	3	3 (1.8%)	6	6 (1.8%)
Hypoglycaemia	3	3 (1.8%)	3	3 (1.8%)	6	6 (1.8%)

[a] Unique within subject and preferred term.
[b] Number of subjects with at least one AE.
Source: List 16.2.7.1

Other Significant Adverse Events – Adverse Events of Special Interest (AESI)

AESI included injection site reactions, immunological reactions, severe hypoglycaemic events and events of hypokalaemia

Injection Site Reactions

There were only a few cases of AESI of injection site reactions and thus no trends were seen for a different distribution between treatment groups for injection site erythema, injection site pain, injection site pruritus and injection site rash. All PTs in the SOC 'General disorders and administration site conditions' containing the phrase 'Injection site' are listed in the table below.

Table 34: Injection Site Reactions (Safety Set)

	Rechon Insulin n=164		Humulin® Regular n=164		Total n=328	
Preferred term[1]	Number of events	Number of patients with event	Number of events	Number of patients with event	Number of events	Number of patients with event
Injection site erythema	1	1 (0.6%)	1	1 (0.6%)	2	2 (0.6%)
Injection site pain	0	0 (0.0%)	1	1 (0.6%)	1	1 (0.3%)
Injection site pruritus	1	1 (0.6%)	1	1 (0.6%)	2	2 (0.6%)
Injection site rash	2	2 (1.2%)	0	0 (0.0%)	2	2 (0.6%)

Source: Table 14.3.1.2
[1]event of a preferred term in SOC General disorders and administration site conditions, counted only once within each patient
n = used as denominator for percentage calculation

Immunological reactions

There were in total 9 AESIs recorded under hypersensitivity in the Insulin Human Rechon group and 6 AESIs in the Humulin Regular group. In the Insulin Human Rechon group, there were 5 AESIs related to the treatment in 3 patients and 4 AESIs not related in 2 patients. Two (2) incidents were not judged as AESI in 2 patients. In the Humulin Regular group, there were 1 AESI related to the treatment and 5 AESIs not related in 4 patients. One (1) incident was not judged as AESI. There were no AESIs involving anaphylaxis, acute bronchoconstrictions, angioedema or acute urticaria.

The occurrence of anti-insulin binding antibodies was the primary objective of this study and is discussed in more detail in section 4.8 of this report.

Table 35: Hypersensitivity reactions (Safety Set)

	Rechon Insulin (n=164)	Humulin® Regular (n=164)
Number of hypersensitivity events	13	6
Number of patients with at least one hypersensitivity event (%)	6 (3.7%)	6 (3.7%)
Source: List 16.2.14		

Hypokalaemia

No AEs with PT hypokalaemia were reported.

Hypoglycaemic episodes

Table 36: Assessment of Hypoglycaemic Episodes According to ADA Criteria (Safety Set)

	Rechon Insulin n=164	Humulin® Regular n=164
All hypoglycaemic events		
Number of events	2700	2217
Number of patients with at least one event n (%)	109 (66.5%)	117 (71.3%)
1) Severe hypoglycaemia		
Number of events	3	3
Number of patients with at least one event n (%)	3 (1.8%)	3 (1.8%)
2) Documented symptomatic hypoglycaemia		
Number of events	2003	1764
Number of patients with at least one event n (%)	101 (61.6%)	110 (67.1%)
3) Asymptomatic hypoglycaemia		
Number of events	582	401
Number of patients with at least one event n (%)	40 (24.4%)	40 (24.4%)
4) Probable symptomatic hypoglycaemia		
Number of events	11	5
Number of patients with at least one event n (%)	3 (1.8%)	2 (1.2%)
5) Relative hypoglycaemia		
Number of events	101	41
Number of patients with at least one event n (%)	23 (14.0%)	14 (8.5%)

Source: Table 14.3.9.1
ADA = American diabetes association

Generalised linear regression analysis between treatments indicated that the rate of hypoglycaemic episodes was higher in the Insulin Human Rechon group than in the Humulin Regular group with a rate ratio and 95% CI of 1.20 (1.14 – 1.27) and a p-value of <0.0001.

Severe hypoglycaemic events

Hypoglycaemic episodes were defined as episodes occurring after first exposure to treatment and no later than 1 day after the last exposure. Each hypoglycaemic episode was classified according to ADA and only those that were classified as severe according to ADA category 1 were reported as AESIs.

There were 6 cases of AESI e.g. hypoglycaemic events that were classified by ADA as severe; affecting 3 patients (1.8%) in each treatment group. All cases of ADA-defined severe hypoglycaemias were also reported as SAEs as they also fulfilled the serious criteria of medically important (2 in the Insulin Human Rechon group and 1 in the Humulin Regular group) and life-threatening (1 in the Insulin Human Rechon group and 2 in the Humulin Regular group). All patients recovered within 1 day without any action taken with study treatment.

Table 37: Cases of American Diabetes Association-defined Severe Hypoglycaemia (Safety Set)

Treatment	Day (duration)	SAE	Severity	Causality	Action taken
Rechon Insulin	150 (1)	Yes	Severe	Possible	None
Rechon Insulin	67 (1)	Yes	Severe	Probable	None
Rechon Insulin	80 (1)	Yes	Severe	Probable	None
Humulin Regular	74 (1)	Yes	Moderate	Probable	None
Humulin Regular	1 (1)	Yes	Severe	Probable	None
Humulin Regular	176 (1)	Yes	Severe	Probable	None

3.3.6.5. Laboratory findings

Laboratory values over time

For clinical chemistry and haematology parameters, there were no apparent trends for the assessed values to change over time from Visit 2 to Visit 5. The assessed values and absolute change from baseline were similar between the treatment groups.

Table 38: Clinical Chemistry and Haematology Assessments at Baseline and Absolute Change at Visit 5 (Safety Set)

	Rechon Insulin n=164		Humulin® Regular n=164	
Parameter (unit) Mean (SD)	Visit 2	Change at Visit 5	Visit 2	Change at Visit 5
Alkaline phosphatase (U/L)	82.1 (22.7)	2.06 (17.3)	81.1 (25.2)	1.32 (17.6)
Alanine aminotransferase (U/L)	28.8 (12.5)	0.0993 (12.1)	28.8 (15.0)	2.66 (19.3)
Aspartate aminotransferase (U/L)	24.4 (20.2)	0.828 (15.0)	24.1 (9.96)	2.89 (13.2)
Total bilirubin (mg/dL)	0.633 (0.311)	0.0100 (0.244)	0.667 (0.302)	0.0161 (0.245)
Creatinine (mg/dL)	0.859 (0.149)	0.0168 (0.106)	0.910 (0.286)	-0.0001 (0.147)
C-reactive protein (mg/dL)	0.286 (0.416)	0.0564 (0.543)	0.386 (0.820)	-0.0793 (0.761)
Gamma glutamyltransferase (U/L)	23.1 (19.3)	2.25 (14.6)	31.8 (37.3)	2.06 (31.7)
Glucose in NaF blood (mg/dL)	183 (74.7)	24.3 (92.1)	186 (86.1)	5.73 (105)
Potassium (mmol/L)	4.46 (0.434)	0.0000 (0.436)	4.41 (0.418)	0.0171 (0.450)
Sodium (mmol/L)	140 (3.76)	-0.809 (3.96)	139 (3.04)	-0.368 (5.44)
Haematocrit (%)	43.9 (3.94)	0.513 (3.30)	44.1 (4.69)	0.899 (4.36)
Haemoglobin (g/dL)	14.1 (1.37)	-0.146 (0.621)	14.3 (1.63)	0.0457 (1.23)
Platelets (10^3/µL)	259 (66.6)	9.20 (53.7)	250 (70.7)	15.1 (52.6)
Erythrocytes (/pL)	4.76 (0.466)	-0.0285 (0.214)	4.76 (0.510)	0.0351 (0.382)
Leukocytes (/nL)	6.14 (1.73)	0.0695 (1.49)	6.13 (1.97)	0.212 (1.44)
Haemoglobin A1c (mmol/mol Hb)	62.6 (12.7)	1.44 (6.99)	63.8 (13.4)	0.739 (9.37)

Source: Table 14.3.4.2.2
Hb = haemoglobin; NaF = sodium fluoride; SD = standard deviation
Mean (SD) is calculated for patients with non-missing data (specified in Table 14.3.4.2.2)

Vital signs

Blood pressure

The mean systolic blood pressure at screening was 129.6 mmHg in the Insulin Human Rechon group and 129.8 mmHg in the Humulin Regular group, which had changed by -1.1 mmHg and -0.5 mmHg, respectively, at Visit 5. The mean diastolic blood pressure at screening was 78.6 mmHg in the Insulin Human Rechon group and 78.1 mmHg in the Humulin Regular group, which had changed by -0.6

mmHg and by -0.2 mmHg, respectively, at Visit 5. At screening, 1 out of 164 patients had CS abnormal systolic and diastolic blood pressure of 168 mmHg and 95 mmHg, respectively. This was a 46-year-old male patient with a medical history of hypertension. No other patients had CS abnormal blood pressure at any other visit.

Pulse

The mean pulse at screening was 74.0 beats/minute in the Insulin Human Rechon group and 73.6 beats/minute in the Humulin Regular group, which had changed by 0.1 beats/minute and by 1.5 beats/minute, respectively, at Visit 5. No patients had CS abnormal pulse at any visit.

Weight

The weight measured at screening is presented in Table 10. The mean change from baseline weight at Visit 5 for females was 0.6 kg in the Insulin Human Rechon group and 0.0 kg in the Humulin Regular group. For males, there was no change from baseline mean weight at Visit 5 in either treatment group (Table 14.3.6.2).

Physical examination

Patients were assessed for general condition, abdomen, heart and lungs at all visits.

There were 3 cases of CS abnormal findings at the physical examinations, which were all recorded under the category 'General condition' (1 patient in the Insulin Human Rechon group at Visit 3), 1 patient in the Humulin Regular group at Visit 4 , and 1 patient in the Rechon Insulin group at Visit 5 . All other assessments in both treatment groups were either normal or NCS abnormal

3.3.6.6. In vitro biomarker test for patient selection for safety

Not available.

3.3.6.7. Safety in special populations

No data on safety in special populations has been provided for this proposed biosimilar product.

3.3.6.8. Immunological events

IgE antibodies

At baseline, circulating IgE levels were 35.0 kU/I in the Insulin Human Rechon group and 37.8 kU/I in the Humulin Regular group, with a change of 0.1 kU/I and 0.8 kU/I compared to the IgE levels at Visit 5 for the treatment groups, respectively.

An ANCOVA assessment did not detect a statistically significant difference between the treatment groups in change from baseline (p-value 0.745 at Visit 5)

Table 39: Level of circulating IgE Antibodies (Safety Set)

IgE antibody level (kU/l)		Rechon Insulin n=164		Humulin® Regular n=164	
		Value	Change from baseline	Value	Change from baseline
Visit 2 (Day 1)	n[a]	164		164	
	Median	35.0		37.8	
	Q1, Q3	13.8, 95.6		17.2, 95.8	
	Min, Max	2, 3423		2, 1217	
Visit 3 (Day 30)	n[a]	159	159	160	160
	Median	34.8	0.00	36.1	-0.700
	Q1, Q3	12.4, 102	-2.40, 2.60	17.1, 94.2	-4.50, 3.35
	Min, Max	2, 4325	-365, 902	2, 741	-476, 290.6
Visit 4 (Day 90)	n[a]	154	154	155	155
	Median	37.3	-0.450	43.2	0.800
	Q1, Q3	12.0, 98.8	-4.20, 4.50	18.0, 96.3	-4.60, 5.80
	Min, Max	2, 4618	-228, 1195	2, 915	-655, 877.5
Visit 5 (Day 180)	n[a]	152	152	152	152
	Median	38.6	0.100	44.9	0.800
	Q1, Q3	12.8, 98.6	-3.55, 8.35	18.4, 103	-5.30, 8.75
	Min, Max	2, 4689	-233, 1540	3.3, 658	-668, 354.5

Source: Table 14.3.4.1.11
n[a] = patients with non-missing data; Q1/Q3 = 1st / 3rd quartile

Table 40: Spearman Correlation of Circulating IgE Antibodies versus Body weight, Fasting Blood Glucose, HbA1c and Insulin Dose at Visit 5 (Safety Set)

Spearman correlation of circulating IgE antibodies vs.:	Rechon Insulin n=164	Humulin® Regular n=164
Body weight [1]	-0.1539	-0.0322
Fasting blood glucose [2]	0.0092	0.0960
Glycosylated haemoglobin A1c [3]	0.1141	0.0752
Insulin dose [4]	0.1004	-0.0747

Source: [1]Figure 14.3.4.1.14; [2]Figure 14.3.4.1.15; [3]Figure 14.3.4.1.16; [4]Figure 14.3.4.1.17

Anti-insulin antibodies

The primary endpoint of this study was to evaluate the proportion of patients having binding antibodies against human insulin. Since it was expected that a large proportion of patients would have anti-insulin antibodies already at baseline, the primary analysis assessed shift from negative at baseline to positive at subsequent visits. For the primary endpoint, PPS was the primary analysis population.

In the PPS, fewer patients were positive for anti-insulin antibodies at baseline in the Insulin Human Rechon group (4.3% of the patients [n=7]) as compared to the Humulin Regular group (7.4% of the patients [n=12]) with only small changes over time. At Visit 5, the difference between the groups was 8.0 percent units more positive patients in the Humulin Regular group than in the Insulin Human Rechon group (95% CI: -13.3, -2.7). The same results were seen in the safety set.

Table 41: Anti-Insulin Binding Antibodies (PPS)

		Rechon Insulin n=164		Humulin® Regular n=164	
			95% CI [a]		95% CI [a]
Visit 2 Day 1	n[a]	163		163	
	Positive	7 (4.3%)	0.9% : 7.8%	12 (7.4%)	3.1% : 11.7%
	Negative	156 (95.7%)		151 (92.6%)	
	95% CI [b]				-3.1 (-8.1 : 2.0)
Visit 3 Day 30	n[a]	158		158	
	Positive	6 (3.8%)	0.5% : 7.1%	12 (7.6%)	3.1% : 12.0%
	Negative	152 (96.2%)		146 (92.4%)	
	95% CI [b]				-3.8 (-8.9 : 1.3)
Visit 4 Day 90	n[a]	154		154	
	Positive	5 (3.2%)	0.1% : 6.5%	11 (7.1%)	2.8% : 11.5%
	Negative	149 (96.8%)		143 (92.9%)	
	95% CI [b]				-3.9 (-8.8 : 1.0)
Visit 5 Day 180	n[a]	150		150	
	Positive	3 (2.0%)	0.0% : 4.6%	15 (10.0%)	4.9% : 15.1%
	Negative	147 (98.0%)		135 (90.0%)	
	95% CI [b]				-8.0 (-13.3 : -2.7)

Source: Table 14.3.4.1.2
[a] = 95% Wald CI; [b] = difference between treatment groups and 95% CI (based on nominal approximation);
n[a] = patients with non-missing data
CI = confidence interval

The individual patients' shift between positive and negative for anti-insulin antibodies is visualized for the Safety Set in the Table below. Out of the patients that were negative for anti-insulin antibodies at baseline, all were still negative in the Insulin Human Rechon group at Visit 5, whereas 3.6% of the patients (n=5) in the Humulin Regular group had become positive for anti-insulin antibodies at Visit 5. Due to the low number of positive samples, 95% CIs were not calculated.

Table 42: Shift in Anti-Insulin Binding Antibodies (PPS)

		Rechon Insulin n=164		Humulin® Regular n=164	
Visit 2, Day 1		Positive	Negative	Positive	Negative
Visit 3 Day 30	n[a]	6	152	12	146
	Negative	1 (16.7%)	151 (99.3%)	2 (16.7%)	144 (98.6%)
	Positive	5 (83.3%)	1 (0.7%)	10 (83.3%)	2 (1.4%)
Visit 4 Day 90	n[a]	6	148	12	142
	Negative	2 (33.3%)	147 (99.3%)	3 (25.0%)	140 (98.6%)
	Positive	4 (66.7%)	1 (0.7%)	9 (75.0%)	2 (1.4%)
Visit 5 Day 180	n[a]	6	144	12	138
	Negative	3 (50.0%)	144 (100%)	2 (16.7%)	133 (96.4%)
	Positive	3 (50.0%)	0 (0.0%)	10 (83.3%)	5 (3.6%)

Source: Table 14.3.4.1.4
n[a] = patients with non-missing data

A sensitivity analysis using logistic regression in the safety set shows a trend for patients in the Humulin Regular group to have a higher probability of being positive for anti-insulin binding antibodies at Visit 3 and Visit 4, compared to patients treated with Insulin Human Rechon. At Visit 5, the difference in probability of being positive for anti-insulin antibodies was statistically significant (p-value 0.0152) in favour of the Insulin Human Rechon treatment. Similar results were seen for the PPS.

Table 43: Odds Ratio for Testing Positive for Anti-Insulin Antibodies (Safety Set)

	Odds ratio (95% CI)[1]	p-value
Visit 3 (Day 30)	0.817 (0.347 : 1.922)	0.6434
Visit 4 (Day 90)	0.759 (0.344 : 1.671)	0.4903
Visit 5 (Day 180)	0.320 (0.128 : 0.803)	0.0152

Source: Table 14.3.4.1.9
[1]odds ratio < 1 implies a higher probability for patients treated with Rechon Insulin to be negative for anti-insulin binding antibodies compared to patients treated with Humulin Regular.

Furthermore, partial Spearman correlations were performed where the change in anti-insulin antibody levels was compared to:

• change from baseline in HbA1c;

• change from baseline in body weight;

• level of fasting glucose; and

• total insulin dose.

At Visit 3, there was a moderate, statistically significant correlation in change from baseline in HbA1c in the Humulin Regular group (0.3630, p-value <0.0001) and a weak positive correlation in total insulin dose in the Insulin Human Rechon group (0.1980, p-value 0.0186). In addition, there was a statistically significant weak negative correlation in change from baseline in body weight at Visit 5 in the Insulin Human Rechon group (-0.1970, p-value 0.0220).

Table 44: Partial Correlation Analysis of Circulating IgE Antibodies (Safety Set)

Parameter compared to change in anti-insulin antibody levels	Visit	Rechon Insulin n=164	Humulin® Regular n=164
Change from baseline in HbA1c (mmol/mol Hb)	Visit 3 (Day 30)	0.0857 (p=0.3125)	0.3630 (p<0.0001)
	Visit 4 (Day 90)	0.0689 (p=0.4239)	0.0849 (p=0.3118)
	Visit 5 (Day 180)	0.0987 (p=0.2548)	0.0574 (p=0.5040)
Change from baseline in body weight (kg)	Visit 3 (Day 30)	-0.0212 (p=0.8033)	-0.0330 (p=0.6925)
	Visit 4 (Day 90)	-0.1105 (p=0.1987)	-0.1329 (p=0.1124)
	Visit 5 (Day 180)	-0.1970 (p=0.0220)	-0.0033 (p=0.9690)
Fasting glucose (mg/dL)	Visit 3 (Day 30)	-0.0546 (p=0.5201)	-0.0404 (p=0.6279)
	Visit 4 (Day 90)	-0.0327 (p=0.7043)	0.0637 (p=0.4483)
	Visit 5 (Day 180)	0.0545 (p=0.5303)	0.0989 (p=0.2483)
Total insulin dose (IU/kg)	Visit 3 (Day 30)	0.1980 (p=0.0186)	0.0089 (p=0.9147)
	Visit 4 (Day 90)	0.1592 (p=0.0631)	0.0124 (p=0.8832)
	Visit 5 (Day 180)	0.1167 (p=0.1776)	-0.0654 (p=0.4461)

Source: Table 14.3.4.1.18

3.3.6.9. Safety related to drug-drug interactions and other interactions

Not applicable.

3.3.6.10. Discontinuation due to adverse events

There were 15 AEs that lead to discontinuation of treatment; 10 AEs in 4 patients in the Insulin Human Rechon group and 5 AEs in 5 patients in the Humulin Regular group.

Table 45: Adverse Events Leading to Discontinuation of Treatment

Patient	Preferred term	SAE/AE	Severity	Causality	Outcome	Action taken
Rechon Insulin						
A2307	Hyperglycaemia	AE	Moderate	Probably	Recovering/ resolving	Yes
A2910	Hyperglycaemia	AE	Moderate	Possibly	Recovered/ resolved	Yes
A3729	Arthralgia Peripheral swelling Hypoesthesia Increased lacrimation Muscle tension	AEs	Moderate	Possibly	Recovered/ resolved	Yes
A2707	Ketoacidosis	SAE	Moderate	Unlikely	Recovered/ resolved	Yes
A2910	Pyrexia Sinusitis	AEs	Mild	Unlikely	Recovered/ resolved Not recovered/ not resolved	Yes
Humulin Regular						
B2901	Unstable diabetes	AE	Moderate	Probably	Recovering/ resolving	Yes
B1331	Urinary tract infection	AE	Moderate	Unlikely	Recovered/ resolved	Yes
B1343	Respiratory tract infection	AE	Mild	Unlikely	Recovered/ resolved	Yes
B2406	Gastric haemorrhage	SAE	Severe	Unlikely	Fatal	Yes
B2819	Suicide	SAE	Severe	Unlikely	Fatal	Yes

Source: List 16.2.7.1

3.3.6.11. Post marketing experience

Not available for the proposed biosimilar product Rechon insulin.

3.3.7. Discussion on clinical safety

Study RCT-004 was an open label study in patients with type 2 diabetes randomized 1:1 to Insulin Human Rechon vs Humulin® Regular reference insulin with a focus on the assessment of safety events related to the originator product Humulin and the proposed biosimilar product Insulin Human Rechon. Following scientific advice given (EMEA/H/SA/2795/1/2014/III) the study duration of 6 months treatment period is considered adequate and the blinding of laboratory staff that performed the antibody analyses is acknowledged. Still, it is noted that the open-label design is prone to bias and the study population of type 1 diabetes patients is known to have a myriad of possible comorbidities and to require concomitant medications to treat primary and secondary symptoms of the disease. Importantly, the study population also maintained their background intermediate or long-term acting insulin treatment during the study, which might hamper a robust interpretation of safety events regarding causality. Both rapid acting insulin IMPs were used as required based on measured glucose levels. The rationale of this design can be followed as this approach resembles a real life setting and is considered an adequate way to follow type 1 diabetic patients. The primary objective of study RCT-004, the assessment of immunogenicity of both treatments, and the respective primary endpoint is appropriate. Also defined safety endpoints as well as the proposed assessment of safety variables, appear appropriate. The study size of 331 participants randomized, and over 90% completing the study, is considered of adequate size to address the primary concern of immunogenicity and insulin tolerance, with blinding of the laboratory staff.

However, it is noted that samples for neutralizing antibodies against insulin were not determined, as technical challenges did not allow to establish a valid assay. As per guideline (EMEA/CHMP/BMWP/32775/2005_Rev. 1) and following provided advice

(EMEA/H/SA/2795/1/2014/III) the potential of detected insulin binding antibodies to influence glycaemic control, insulin requirements and safety, especially local and systemic hypersensitivity reactions should be discussed. Upon request, the applicant has provided comparative data for the same subjects while not positive for antibodies and to subjects without insulin antibodies during study RCT-004. Furthermore, the applicant has confirmed that no pattern was identified in association with the positive anti-insulin antibody detection regarding glycaemic control, insulin requirements or safety. Two substantial protocol amendments were conducted, which included the merging of information in the two local CSP versions into a single global version and the change of the primary variable The applicant has confirmed that the change in assays (amendment 2) was implemented before any results were available and that all samples from study RCT-004 were analysed with the electrochemiluminescence immunoassay method for insulin binding antibodies. Outline reasons for change in assay can be followed.

Reasons for study discontinuation appear rather balanced across groups and do not give reason of concern. A number of major protocol deviations is reported. The applicant has provided a list and summary of major protocol deviations of study RCT-004 separated by treatment arms and no pattern of concern appears evident from the provided listing. Further, one subject was withdrawn and excluded from the PPS due to lack of compliance in agreement between investigator and Sponsor. By the time of exclusion, the patient had not reported any adverse event. No concerns arise. With respect to imbalanced gender distribution across treatment arms the applicant was asked to list results for anti-insulin antibodies and SAEs in female and male subjects treated with Rechon and Humulin. Reassuringly, none of the subjects who experienced SAE has anti-insulin antibodies. In study RCT-004 for both treatment groups only a minimal change in required bolus insulin was recorded, which was comparable for both treatment groups and independent whether subjects used intermediate-acting insulin or long/ultralong-acting insulin as background insulin.

Adverse events

The provided summary of adverse events does not indicate any major imbalances across both treatment groups regarding number of events and patients for AEs, serious AEs, AEs leading to discontinuation or study withdrawal. No substantial difference is apparent for PTs as most of them were reported in single patients. The highest difference in patients with event is reported for PTs hyperglycaemia and arthralgia with each PT reported in 3 patients treated with Insulin Human Rechon and no patient treated with Humulin. The number of patients with at least one adverse event does also not indicate any imbalance between treatment arms (28% and 26.2% of patients treated with Rechon and Humulin, respectively). All events of hyperglycaemia were of non-serious severity and no other clinically significant abnormalities were identified for the time of the hyperglycaemic event. It is acknowledged that hyperglycaemia can be caused by a multitude of possible reasons and that study RCT-004 was not designed to formally describe any possible difference in efficacy. The in total 3 events that were categorised as PT hyperglycaemia in subjects treated with Insulin Human Rechon do not indicate a substantial imbalance between treatment arms.

Two deaths were reported during the study, both in the Humulin treatment group. Narratives are provided and causality in both cases appears unlikely. All serious adverse events that were related to treatment were events of hypoglycaemia with 3 events in each of the treatment groups. All other serious adverse events were single events without any pattern indicating increased risk for either of the two treatments. Less subjects reported more events of hypoglycaemia when treated with Insulin Human Rechon compared to Humulin during study RCT-004 (2700 and 2217 events in 109 (66.5%) and 117 (71.3%) subjects, respectively. Thus, no clear trend that would indicate an increased risk of hypoglycaemia can be concluded for either of the two treatments. With respect to AESIs, serious

events of hypoglycaemia related to study treatment and considered an adverse event of special interest were balanced across groups (3 events each), none of the events required further action (study drug continued) and all of the events resolved within one day.

No pattern of concern is concluded for events of hypersensitivity as the number of patients with that reported a hypersensitivity event was identical in both groups (n=6), no anaphylaxis reaction was reported, all events resolved and none of the events required further action to be taken. No event of hyperkalaemia was reported and injection site reactions reported as AESI were rare and without worrisome pattern for either of the rapid insulin treatments. The majority of all measures for chemistry and haematology measures are well balanced between treatment groups at baseline and follow a comparable change from baseline until day 180 (visit 5). Haemoglobin A1c and creatinine are important measures to evaluate the stability of type 1 diabetes and both measures do not indicate any relevant difference between both treatment groups. Also, no crucial differences in changes during the study period were observed for vital signs and physical examinations between the reference product and the comparator. Thus, no concerns arise regarding biosimilarity. No major imbalance is identified for adverse events leading to study discontinuation.

Immunogenicity

No relevant differences in circulating IgE levels are detected between treatment groups and no clinically relevant correlation with either body weight, fasting blood glucose, HbA1c or insulin dose was identified. Levels of circulating IgE Antibodies do not appear associated with any specific adverse event/immunological reaction. It is noted that several factors might have an impact on general IgE levels (e.g. ongoing infections, allergies, personal traits) and any direct link to the rapid acting insulin would be difficult to establish.

More subjects in the Humulin group were positive for anti-insulin antibodies at baseline (7.3% and 4.3% for Humulin and Insulin Human Rechon, respectively). A mild imbalance for lower immunogenicity in favour of insulin Human Rechon was also observed throughout all study visits (at day 180: 9.9% and 2% of subjects had anti-insulin antibodies in the Humulin and Insulin Human Rechon group, respectively). Of relevant note is that from the 144 subjects that were negative at baseline and were tested at the final study visit after 180 days (PPS), no subject was positive for anti-insulin antibodies in the study group treated with Insulin Human Rechon. In turn, from the 138 subjects that were negative at baseline and were tested at the final study visit after 180 days (PPS), in total 5 subjects were positive for anti-insulin antibodies in the study group treated with Humulin. Thus, data from study RCT-004 indicate that subjects treated with the proposed biosimilar product appear to have a lower tendency to develop anti-insulin antibodies compared to the reference product. All patients also received background intermediate or long-acting insulin with potential immunogenic potential that could have contributed to upcoming antibodies. No changed background insulin treatment (i.e. intermediate or long-acting insulin) or change in the method of insulin administration (continuous vs multiple SC injections) was evident for any of the subjects that was identified with anti-insulin binding antibodies. Currently, a conclusion regarding differences in patients with positive binding antibodies does not seem robust from the data at hand. Similarly, conclusions regarding statistically significant correlations for change from baseline in HbA1c (Humulin day30), total insulin dose (Insulin Human Rechon day30) and change from baseline in body weight (Insulin Human Rechon day180) appear not fully conclusive, as also no general pattern could be identified (single occasions with positive results). No concerns arise from reported results for the treatment with Insulin Human Rechon regarding anti-insulin binding antibodies.

Study RCT-001

No crucial imbalances in reported AEs are evident from study RCT-001 in healthy subjects that received twice 0.3 IU/kg in a cross over design. No serious adverse events or discontinuations were

reported and there were no major changes in laboratory parameters and no clinically significant values were reported. Reported changes in vital signs, ECG and physical examinations during the study do not appear critical. It is noted that in total 4 subjects had to be treated for a clinically significant hypokalaemia (3 subject following Rechon Insulin and 1 subject following Humulin treatment). Considering also results from chemistry evaluation the amount of subjects with low potassium appears balanced between both treatment groups.

3.3.8. Conclusions on clinical safety

In conclusion, safety data are principally considered adequate for the assessment of biosimilarity. No major imbalances between the reference product and the proposed biosimilar treatments were reported regarding quantity and quality of safety events during studies RCT-004 and RCT-001. Also, no concerns arise from reported results for the treatment with Insulin Human Rechon regarding anti-insulin binding antibodies. Still, it is noted that subjects treated with the proposed biosimilar product appear to have a lower tendency to develop anti-insulin antibodies compared to the reference product, at least with respect to results presented for study RCT-004.

3.4. Risk management plan

3.4.1. Safety specification

Summary of safety concerns

The applicant provided a revised version 1.1 of the RMP and proposed the following summary of safety concerns in the RMP:

Table 46: Summary of safety concerns

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

3.4.1.1. Discussion on safety specification

Part II, Module SI has been updated accordingly, and the referenced RMP is now version 6.1.

. In the revised RMP Version 1.1 severe hypoglycemia is no longer considered a significant safety concern in the Risk Management Plan, aligning with the current RMP of the reference product. The risk can be appropriately managed in clinical practice through routine risk minimisation measures and is sufficiently labelled in the SmPC/PL

3.4.1.2. Conclusions on the safety specification

Having considered the data in the safety specification

It is agreed that the safety concerns listed by the applicant are appropriate.

3.4.2. Pharmacovigilance plan

The current pharmacovigilance plan submitted by the applicant is as follows:

Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

- Specific adverse reaction follow-up questionnaires

Not applicable.

- Other forms of routine pharmacovigilance activities

On a monthly basis, the AE/product complaint (PC) committee will review AEs to detect increased frequency trends potentially related to lot-specific PCs. The AE/PC databases will be queried and reviewed for potential drug-event combinations (DEC) that might indicate a manufacturing-related event. Any such DECs or trends would be further reviewed by safety physician and other personnel as indicated.

Summary of planned additional PhV activities

Table 47: On-going and planned additional pharmacovigilance activities

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities *(by the competent authority)*				
None				

The proposed pharmacovigilance plan has been updated in line with the requested changes to the safety concerns and is formatted in accordance with current RMP requirements.

Overall conclusions on the PhV plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

The PRAC Rapporteur also considered that routine PhV remains sufficient to monitor the effectiveness of the risk minimisation measures. No further changes to the pharmacovigilance plan are needed at this point in time.

3.4.3. Summary of post authorisation efficacy development plan

Not applicable.

3.4.4. Risk minimisation measures

The safety information in the proposed product information is aligned to the reference product.

Routine risk minimisation measures

The applicant has aligned the risk minimisation measures to the ones of the reference product except for one risk that does not apply to Insulin Human Rechon: 'Product differentiation to address the risk', for the following reasons:

- Insulin Human Rechon exists only as a fast-acting insulin (no risk of confusion between slow-acting and fast-acting insulin);
- Insulin Human Rechon is available in cartridges which are only suitable for subcutaneous injections using a reusable pen.

In addition, The applicant has aligned the safety information in the proposed product information to the reference medicinal product and has implemented the recommendations related to units in the product information (SmPC, Labelling and Package Leaflet). To avoid medication errors that were reported for high strength and fixed-combination insulin products due to the use of abbreviation of units (Risk minimisation strategy for high-strength and fixed combination insulin products - Addendum to the good practice guide on risk minimisation and prevention of medication errors [EMA/686009/2014]), the units of the product have been spelled in full and using lower case ("units/ml" instead of IU/mL) in order to avoid any confusion (a 11-fold dosing error could be done if 1IU was seen as 11U).

Additional risk minimisation measures

Not applicable.

Overall conclusions on risk minimisation measures

The PRAC Rapporteur having considered the data submitted was of the opinion that:

In line with the reference product the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s).

3.4.5. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 1.1 with data lock point of 30-05-2024 and final sign off date of 27-05-2024 is acceptable.

3.5. Pharmacovigilance

3.5.1. Pharmacovigilance system

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

3.5.2. 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.

4. Biosimilarity assessment

4.1. Comparability exercise and indications claimed

The applicant applies for the following indication that is identical to the approved reference product Humulin ® Regular as stated in the respective SmPC: *"the treatment of patients with diabetes mellitus who require insulin for the maintenance of glucose homeostasis."*

Quality

At quality level, an extensive analytical comparability study has been performed. However, based on the totality of analytical data provided, insulin Rechon finished product can be considered comparable to Humulin R reference product.

Non-clinical

In Module 4, an in vitro biosimilarity approach was submitted in which Insulin Human Rechon and its comparator Humulin® Regular from Eli Lilly were examined for their potential to bind and activate the insulin receptor and to trigger insulin-specific metabolic changes in insulin-sensitive cell cultures.

Clinical

Two clinical studies were submitted to support the claim of biosimilarity, one combined PK/PD euglycaemic clamp study in healthy volunteers (Study Code RCT-001) and one safety and immunogenicity study in patients with T1DM (Study Code RCT-004). The scope of both studies were in line with the respective guideline (EMEA/CHMP/BMWP/32775/2005_Rev. 1) and the clinical developmental program principally followed guidance as provided by the CHMP (EMEA/H/SA/2795/1/2014/III) and the Swedish national authority (MPA; Dnr 161:2010/511053).

4.2. Results supporting biosimilarity

Quality

Overall, the chosen panel of analytical methods seem suitable for the intended use and including also orthogonal analytical methods appropriate to detect differences between the test and the reference, if any. Structural comparability of insulin drug substance samples isolated from the Insulin Human Rechon DP and the reference product Humulin R was studied using physico-chemical state-of-the-art analytical methods. Similarity between the test and the reference product with regard to primary, secondary and tertiary structure, molecular mass and size distribution is postulated and can be considered overall justified by the provided data. In the forced degradation study, one Insulin Human Rechon DP batch was compared against one Humulin R batch. Stress, as impacted by variation of pH, temperature, oxidation/reduction, light, freeze/thaw and mechanical stress has been investigated using RP-LC/MS and SEC-MALS methods. Mainly, the investigated stressors induce comparable elevated levels of deamidation and oxidation as well as increased levels of HMWS (insulin aggregates) and LMWS (probably insulin monomer and dimer).

Non-clinical

The applicant submitted comparative in vitro PD studies in which biosimilarity between Insulin Human Rechon and its comparator Humulin® Regular from Eli Lilly were studied in receptor binding assays as well as in cellular in vitro assays. The latter comprise assays in insulin-sensitive cells, and by studying metabolic activation of the cells by the different insulin products. Specifically, metabolic activation was examined by measuring glucose uptake, lipogenesis and inhibition of lipolysis of the cells when the

different insulin products were added to the cell cultures. The gathered results in these in vitro experiments support the biosimilarity claim between Insulin Human Rechon and its comparator Humulin® Regular.

Clinical

Biosimilarity in PK/PD was evaluated in healthy subjects in the scope of a double-blind, cross-over, euglycaemic clamp study. Results for primary measures regarding PK (AUC0-10 and Cmax) as well as for PD (GIR-AUC0-10 and GIRmax) support the conclusion of biosimilarity as the CIs (90% for PK and 95% for PD) of the geometric mean ratios were within the pre-defined bioequivalence acceptance range (0.8-1.25): 90% CIs 0.933 - 0.984 for AUC(0-10 h) and 0.914 - 1.110 for Cmax, 95% CIs 0.922 - 1.064 for GIR-AUC(0-10 h) and 0.960 - 1.176 for GIRmax. In conclusion to study RCT-001, the insulin concentration as well as the required GIR to compensate for reduced blood glucose levels all principally support the claim of biosimilarity with currently available data. No imbalance in safety measures was observed during the study in healthy subjects. Further, no major imbalances between the reference product and the proposed biosimilar treatments were reported regarding quantity and quality of safety events in type 1 diabetes patients during study RCT-004.

4.3. Uncertainties and limitations about biosimilarity

Quality

Comparability on analytical level currently can be finally concluded.

Non-clinical

The non-clinical comparability included several studies (in vitro pharmacodynamic studies as well as in vivo toxicology study) to support the biosimilarity of Insulin Human Rechon to the reference medicinal product Humulin® Regular form Eli Lilly. Although the provided in vitro study results show good comparability between Insulin Human Rechon and Humulin® Regular in terms of binding characteristics and pharmacological effects (gluose uptake, lipogenesis, inhibition of lipolysis), the documentation on how the standard deviations of all controlled values in Study 2021-10-28-Rechon were calculated is not fully clear.

Clinical

In-house validation of assay performance (i.e. demonstrating reproducibility and precision) to detect insulin and C-peptide is only sparsely reported. During the euglycaemic clamp study healthy subjects were clamped at a glucose level of 6 mmol/L, but fasting levels were mostly below this value (N=29 <6mmol/L, N=4 ~6mmol/L, N=8 >6mmol/L). As a consequence, it is unclear whether the conclusion on similarity was potentially favoured by variable endogenous insulin levels as the high clamp level causes the clamp study results to be a mixture of the effects of both endogenous and administered insulins. Furthermore, the applied infrequent sampling frequency of intervals of 30min around Tmax is considered inadequate to reliably estimate Cmax. Nevertheless, the levels around Cmax are considered sufficiently stable such that Cmax is accepted as sufficiently covered, though Tmax may not be fully covered and this aspect remains as uncertainty. Conclusions on biosimilarity cannot presently be sufficiently supported based on available data from study RCT-001. From reported data it cannot be excluded that the proposed biosimilar Insulin Human Rechon might have a slightly shorter half-life compared to the originator Humulin Insulin.

Insulin Human Rechon was reported with a higher inter-individual CV% for GIRmax and also AUC-GIR compared to Humulin, indicating that a more variable degree of glucose infusion was required across

subjects to keep the target glucose level when treated with Insulin Human Rechon. This observation indicates that a more variable degree of glucose infusion was required across subjects to keep the target glucose level when treated with Insulin Human Rechon. The GIR algorithm as applied in study RCT-001 was amended during the study and apparently did not cover all glucose fluctuations that occurred during the study. The overall number of out-of-algorithm changes in GIR adjustment appears high and the impact of such deviations on biosimilarity assessment remains uncertain. Data smoothing was applied prior calculation of both primary PD variables (AUC-GIR(0-10 h) and GIRmax). The smoothing factor was determined during a blind data review before database lock, which might have supported conclusions on similarity, depending on the chosen smoothing factor. Required sensitivity analyses for GIRmax using a smoothing factor of 0.05 or 0.1 (i.e. less smoothing compared to the original analysis) gave results not meeting the bioequivalence criteria. A rather large number of haemolysed samples (8.8% of all samples provided for insulin and C-peptide analysis) were excluded from study RCT-001 from the analysis. Exclusion of haemolysed samples appears justified given the bad quality of samples, but an uncertainty nevertheless remains concerning the total extent of haemolysed samples.

Fewer of the subjects treated with the proposed biosimilar product developed anti-insulin antibodies (i.e. were negative at baseline and positive during the study) during study treatment of study RCT-004 compared to subjects treated with the reference product. The total number of subjects with antibodies as well as reported differences between treatments are small (5 subjects that were negative at baseline were positive after treatment with the reference product at day 180, none was positive after treatment with the proposed biosimilar). Furthermore, respective conclusions do not appear very sensitive from the data at hand as all patients also received background intermediate or long-acting insulin therapy with potential immunogenic potential that could have contributed to upcoming antibodies. Importantly, no safety concerns arise from reported results for the treatment with the proposed biosimilar.

4.4. Discussion on biosimilarity

An extensive analytical comparability study has been performed. Based on the totality of analytical data provided, Insulin Human Rechon finished product manufactured most recently in 2019 can be considered comparable to Humulin R reference product from a quality perspective.

Submitted non-clinical data largely support a conclusion on biosimilarity between the proposed biosimilar product and the reference insulin product.

Regarding clinical data, the glucose clamp level applied in study RCT-001 currently obstructs the conclusion on biosimilarity. Reliable evidence on the proposed biosimilarity of Insulin Human Rechon to Humulin Insulin is required, before equivalence on clinical PK/PD level can be concluded. Observed differences in the immunogenic profile, with a higher rate of subjects developing anti-insulin antibodies in the group treated with the reference product, do not conclude safety concerns for the treatment with the proposed biosimilar. Also, biosimilarity is not directly questioned by results as the total numbers of subjects with antibodies as well as reported differences between treatments are small (5 subjects that were negative at baseline were positive after treatment with the reference product at day 180, none was positive after treatment with the proposed biosimilar). Of note, respective conclusions from the safety study RCT-004 do not appear very sensitive as all patients also received background intermediate or long-acting insulin therapy with potential immunogenic potential that could have contributed to upcoming antibodies.

In conclusion, even though available data on quality, non-clinical and clinical level do not directly preclude a possible biosimilarity between products, critical uncertainties remain as the presently available clinical data from study RCT-001 are still not sufficiently compelling to establish PK/PD

equivalence of Insulin Human Rechon versus the reference. Reliable evidence on the proposed biosimilarity of Rechon Insulin to Humulin Insulin is required, before equivalence on clinical PK/PD level can be concluded.

4.5. Extrapolation of safety and efficacy

No extrapolation to other indications is requested for this biosimilar application.

4.6. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Insulin Human Rechon is considered not biosimilar to Humulin® Regular. Therefore, a benefit/risk balance comparable to the reference product cannot be concluded.

5. List of outstanding issues to be addressed in an oral explanation and/or in writing

5.1. Quality aspects

None

5.2. Non-clinical aspects

None

5.3. Clinical aspects

Major objection

1. It cannot be excluded that endogenous insulin might have interfered with primary endpoint measures of study RCT-001. Therefore, PK/PD equivalence of Insulin Human Rechon versus the reference cannot be reliably concluded based on available data. Compelling evidence is required to demonstrate equivalent PK/PD.

Other concerns

None

5.4. Risk management plan

None