EDA Assessment Report for Biological Medicinal Product

(Scientific Discussion)

Bonosome r-DNA

Date: December 2023

Unit: Technical Assessment Unit

Assessment report

Bonosome r-DNA

Administrative information:

Invented name of the medicinal product:	Bonosome r-DNA
INN (or common name) of the active substance(s):	Teriparatide (r-PTH 1-34)
Marketing Authorization holder	NA
Applied Indication(s):	- Treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture. In postmenopausal women, a significant reduction in the incidence of vertebral and non-vertebral fractures but not hip fractures has been demonstrated. - Treatment of osteoporosis associated with sustained systemic glucocorticoid therapy in women and men at increased risk for fracture
Pharmaceutical form(s) and strength(s):	-Solution for injection in prefilled syringe for subcutaneous administration. -Strength: 20 mcg rhPTH 1-34, teriparatide
Route of administration	Subcutaneous injection in the thigh or abdomen

List of abbreviations

ADAs	Anti-drug antibodies
APS	Aseptic Process Simulations
AE	Adverse effect
AUC0-∞	Area under the plasma concentration versus time curve to infinity
AUCext extrapolated	Percent of area under the plasma concentration versus time curve to infinity
BMD	bone mineral density
CQAs	Critical quality attributes
CTD	Common technical document
CMO	Contract manufacturing organization

CD	Circular dichroism
cAMP	Cyclic adenosine monophosphate
CRO	Clinical research organization
CV	Coefficient of variation
CIs	Confidence interval
CL/F	apparent total clearance
Cmax	Maximum concentration
DP	Drug Product
EU	European Union
ECG	Electrocardiogram
GMP	Good manufacturing practice
G	Gauge
HEK	Human embryonic kidney
INN	the international non-proprietary name
IPCs	In-Process Controls
IEF	Iso-Electric Focusing
LC- ESI-MS	Liquid chromatography-electron spray ionization-mass spectrometry
LC-MS/MS	Liquid chromatography coupled by tandem mass spectrometry
LS	least square
lz	apparent terminal elimination rate constant
MCB	Master cell bank
micro-CT	microcomputed tomography
Max	Maximum
Min	Minimum
PD	Pharmacodynamic parameter
PTH-R1	parathyroid hormone-receptor-1
PD	Pharmacodynamics
PK	Pharmacokinetics
QTPP	Quality Target Product Profile
r-DNA	Recombinant DNA
RP-HPLC	Reversed phase high performance liquid chromatography
rh-PTH 1-34	Recombinant human Parathyroid Hormone
RNS	Rigid Needle Shield
SOPs	Standard Operating Procedures
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylmide gel electrophoresis
SEC-UV	Size exclusion chromatography-ultra violet
SC	Subcutaneous
SD	Standard deviation
TEAEs	Treatment Emergent Adverse Event
T last	time to last measurable concentration
Tmax	time to maximum concentration
t½	apparent terminal elimination half-life
TW	Thin wall

USP	United states pharmacopeia
US	United States
WB	Western blot
WHO	World health organization
Vz/F	apparent distribution volume
µg	microgram
µl	microliter

Dossier initial submission and evaluation process.

- The product was submitted for registration via 343/2021 ministerial decree.
- The dossier evaluation by the registration administration units was started on 7.4.2022 after providing all the required documents according to the Checklist for documents of new biological products registration file.
- Full CTD along with detailed SOPs were provided.

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1. General introduction about the product including brief description of the AI, its mode of action and indications

- Osteoporosis, as defined by World Health Organization, is a systemic disease of the skeleton characterised by low bone mineral density (BMD) and micro-architectural deterioration of bone tissue with consequent increased bone fragility that predisposes to fracture risk. Due to the silent progression of bone structure degeneration, osteoporosis diagnosis often follows a painful fracture event.

-The diagnosis of osteoporosis is established by means of bone densitometry or by the presence of a fragility fracture. Any bone may be affected; although the skeletal sites most prone to fracture include proximal femur (hip), vertebrae (spine), and distal forearm (wrist). Osteoporotic fractures lead to pain and occasional disability. More importantly, they increase mortality.

- Osteoporosis is commonly experienced in postmenopausal women due to declining oestrogen-levels. However, osteoporosis can also occur in both sexes as a side effect of prolonged treatment with glucocorticoid medications. Glucocorticoid-induced osteoporosis may be responsible for up to 20% of all osteoporosis cases. Fractures, primarily hip fractures, decrease a patient's quality of life by increasing pain, medical costs, morbidity, and mortality.

About the product

- Recombinant human teriparatide (rhPTH (1-34)) is the international non-proprietary name (INN) for the biologically active 34-amino acid N-terminal fragment of the 84-amino acid native parathyroid hormone, PTH (1-84). Synthetic and genetically engineered versions of teriparatide both exist, sharing identical affinity for the parathyroid hormone (PTH) surface receptors as well as possessing the same biological activity.
- The active substance in Bonosome r-DNA, biosimilar, is produced in Hansenula polymorpha using recombinant DNA technology.
- Recombinant teriparatide contains no amino acid substitutions or chemical modifications and differs from the synthetic peptide only in its method of production and purification. Recombinant teriparatide contains no glycosylation or other post-translational modifications.
- Endogenous PTH (1-84) is the primary regulator of calcium and phosphate metabolism in bone and kidney. Physiological actions of PTH include stimulation of bone formation by direct effects on bone forming cells (osteoblasts) indirectly increasing the intestinal absorption of calcium and increasing the tubular reabsorption of calcium and excretion of phosphate by the kidney.
- The molecular effects of teriparatide are mediated by the parathyroid hormone-receptor-1 (PTH-R1), a G– protein-dependent membrane receptor expressed by osteoblasts and renal tubular cells. Teriparatide has similar affinity for the PTH-R1 as PTH (1– 84). PTH signaling results in the activation of genes important for the functions of mature osteoblasts, increases in osteoblast number, decreases in the apoptotic rate of osteoblastic cells, and increases in their bone-forming activity. The net result is an increase in the number of active osteoblasts, a decrease in osteoblast apoptosis and probably a recruitment of bone lining cells as newly formed osteoblasts, which are followed by increasing bone strength, mass and diameter and bone structural integrity, as well as increasing levels of biochemical markers of bone turnover (both formation and resorption markers) in serum and urine (Blick et al., 2008).

-The therapeutic indications, posology and route of administration proposed for Bonosome r-DNA are identical to those for Forteo, to which similarity is claimed.

Forteo is authorised for the following therapeutic indications:

- Treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture. In postmenopausal women, a significant reduction in the incidence of vertebral and non-vertebral fractures but not hip fractures has been demonstrated.
- Treatment of osteoporosis associated with sustained systemic glucocorticoid therapy in women and men at increased risk for fracture.
- The recommended dose is 20 µg administered once daily by subcutaneous (sc) injection in the thigh or abdomen. The maximum total duration of treatment with teriparatide should not exceed 24 months. The 24- month course of teriparatide should not be repeated over a patient's lifetime.

Forteo (20 µg/80 µL solution for injection) is supplied in a pre-filled disposable pen containing 28 doses. Bonosome r-DNA (biosimilar teriparatide, 20 µg/80 µL solution for injection) is supplied in a single dose prefilled syringe.
Bonosome r-DNA (teriparatide 20 µg/80 µL solution for injection) has been developed as a biological medicinal product and is claimed to be biosimilar to Forteo which contains recombinant human teriparatide (rhPTH (1-34)) as active substance (20 µg/80 µL solution for injection).

The development program of Bonosome r-DNA has in general been aligned to the respective EDA guidance, in particular:

- GUIDELINE FOR REGISTRATION OF BIOSIMILAR PRODUCTS IN EGYPT; March 2020

2. Quality aspects:

1.2.1 Introduction

The active substance of Bonosome r-DNA product recombinant human teriparatide (rhPTH (1-34)), which is the biologically active 34-amino acid N-terminal fragment of the 84-amino acid native parathyroid hormone. Recombinant human teriparatide (rhPTH (1-34)) belongs to a class of anti- osteoporosis drugs, the so-called "anabolic" agents.

The finished product (Bonosome r-DNA) is presented as solution for injection containing 20 micrograms/80 microliters (20 µg/80 µL) of Teriparatide as active substance. Each prefilled syringe contains 80 microliters of solution.
Other ingredients are glacial acetic acid, mannitol, metacresol, sodium acetate trihydrate, hydrochloric acid, sodium hydroxide, water for injection.

The product is available in a glass barrel made from borosilicate glass type I, with a type 27 G ½ inch stainless steel needle with RNS (rigid needle shield), grey/black (halobutyl) rubber plunger and a 0.5 ml plunger rod.
Bonosome r-DNA 20 micrograms/80 microliters solution for injection (INN of active substance: teriparatide) has been developed as an intended biosimilar medicinal product of FORTEO® (Eli Lilly).

1.2.2Drug Substance (Active ingredient)

- **General information**

Bonosome r-DNA drug substance is teriparatide, an engineered variant produced in Hansenula polymorpha. using recombinant DNA technology, consisting of the 34 N-terminal amino acids of native human parathyroid hormone. Recombinant human teriparatide (rhPTH (1-34)) is retaining full biological activity.
The theoretical monoisotopic mass of teriparatide is 4117.7 Dalton (C181H291N55O51S2).
The amino acid sequence is:

SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHNF

Teriparatide contains no glycosylation or other post-translational modifications.

Biological activity:

Teriparatide (rhPTH(1-34)), is a recombinant 1-34 N-terminal fragment of endogenous human parathyroid hormone, which is critical for G-protein linked stimulation of adenylate cyclase that catalyzes the formation of second messengers such as cAMP. The 84-amino acid parathyroid hormone (PTH) stimulates the bone formation by direct effects on bone-forming cells (osteoblasts), indirectly increasing the intestinal absorption of calcium and increasing the tubular re-absorption of calcium and excretion of phosphate by the kidney. The amino terminus is critical for G-protein linked stimulation of adenylate cyclase that catalyses the formation of second messengers such as cAMP that activates the desired biological effects by phosphorylation of critical intracellular proteins. The biological activity of teriparatide is determined using a cell-based assay.

- **Manufacture, process controls and characterization:**

Manufacturer:
The active substance manufacturing take place at Rhein Minapharm Biogenetics Mina Street - Industrial Zone # 3/A2 10th of Ramadan City – Egypt in accordance with good manufacturing practice.

Description of Manufacturing Process and Process Controls

-The manufacturing process for PTH comprised of MCB vial thaw, inoculum expansion, and inoculation of production fermenter and target protein then further filtration and purification of the drug substance.
- All process steps and materials controls are well described.

Control of Materials

-Sufficient information on raw materials used in the active substance manufacturing process has been submitted.
-All raw materials are sourced from qualified suppliers. Raw materials are received, identified, tested and released according to written Standard Operating Procedures (SOPs) as required by cGMP.
-Materials used in the manufacture of drug substance are tested internally and accepted on the basis of relevant pharmacopeia testing methods & Supplier's Certificate of Analysis with reference to internal specifications.

Controls of Critical Steps and Intermediates

-critical process parameters for manufacturing steps were risk based chosen for assessing each process step and identifying the critical parameters to achieve the required product quality, purity, and protein expression.

Process Validation

The Bonosome r-PTH1-34 active substance manufacturing process has been validated adequately. Consistency in production has been shown on different batches.

All acceptance criteria for the critical operational parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the manufacturing process consistently produces Bonosome r-PTH1-34 active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Manufacturing Process Development.

The initial drug substance manufacturing process has been developed at research and development labs at Minapharm Pharmaceuticals.

The non-clinical material was supplied from a pre-commercial scale that was also used for the initial biosimilarity evaluations.

The process was then transferred to a European CMO for clinical material manufacturing, to supply the proposed clinical study and stability studies.

The process was up scaled to the final commercial scale at Rhein Minapharm Biogenetics. Three batches of commercial manufacturing scale were produced for process validation and stability studies.

- **Characterization**

-The characterization of Bonosome r-DNA drug substance includes the determination of physicochemical properties, biological activity, purity, impurities and quantity of the drug substance, as well as the primary and higher order structures.

-The presented data confirm the expected structural and functional characteristics of PTH 1-34

-Product-related impurities are properly monitored during manufacturing, release and stability testing.

In addition, the process-related impurities were evaluated during commercial manufacturing to demonstrate that the manufacturing process provides adequate removal of such impurities.

- **Specification**

The release specification for the active substance comprises tests for identity, purity and impurities, potency, quantity, microbiological attributes and general attributes.

The specification has been prepared in line with the requirements of requirements of ICH guideline Q6B. The specification takes into account the critical quality attributes (CQAs) of the active substance that can affect the safety and efficacy of the product, and defines the acceptable range for the physicochemical and biological characteristics of the active substance within the context of the wider control strategy.

- **Analytical Procedures**

-Tests are performed using compendial analytical test methods, where applicable. Pharmacopoeial methods and acceptance criteria references refer to the current versions of the pharmacopoeias and their supplements. Quality control is maintained by adherence to cGMP requirements and ICH guidelines.

- All the non-compendial method were adequately described and appropriately validated in accordance to ICH guidelines.

- **Batch analysis**

Commercial batches representing process validation analyses data were submitted and their results comply with specification sheet and defined acceptance criteria.

- **Reference Standards or Materials**

- Ampoules of the WHO International Standard for Parathyroid hormone and in-House Secondary Reference Standard has been employed in the analysis of the active substance and finished product for release and stability testing during development. Both reference standards are appropriately identified and characterized.
-The in-house reference standard quality was evaluated through a collaborative study by a set of release tests according to the scope of usage & other additional characterization tests.

- **Container closure system**

-Bonosome r-DNA drug substance is packaged in containers composed of polyethylene terephthalate copolyester, glycol modified (PETG) bottle and high density polyethylene (HDPE) closure. Both are compliant with USP Class VI requirements.
- The bottle and the closure are supplied pre-sterilized and are non-pyrogenic.
-The container closure components are compatible with Bonosome r-DNA drug substance, and suitable for storage of Bonosome r-DNA drug substance under the intended -80 ± 10ºC temperature condition.

- **Stability of drug substance**

The stability studies are conducted at long-term storage conditions as well as under accelerated conditions using Bonosome active substance batches that were manufactured according to the intended commercial manufacturing process at the intended commercial site.-Real time, real condition stability data of active substance for stored in a representative container closure system were provided. Data under accelerated conditions according to the ICH guidelines were provided.

-stability data of real time, stress conditions were provided.
-Suggested Storage Conditions of the active substance: - 80°C ± 10 °C

-required shelf life for the active substance is 2 years

2.2.3 Drug product:

-Description and Composition of the Drug Product:

Bonosome r-DNA 20 micrograms/80 microliters (20 µg/80 µL) solution for injection is a clear, colourless solution. Each prefilled syringe contains 80 µL of solution.

The drug product is packaged in Glass barrel made from borosilicate glass type I, according to USP, EP, assembled with type 27 G ½ inch stainless steel needle with RNS (rigid needle shield Stelmi® GS 4800 grey. (Halobutyl) rubber plunger.

- Pharmaceutical Development

Components of drug product

-The active ingredient Bonosome r-DNA is an engineered variant of native human parathyroid hormone (hPTH) retaining its N-terminal subunit biological activity. It is a polypeptide consisting of amino acids 1-34 of hPTH. Drug substance.

-The excipients of the finished product are glacial acetic acid, sodium acetate trihydrate, mannitol, metacresol, water for injections.

- Formulation Development

The composition of the drug product was selected to match exactly the formulation of the reference products manufactured by Eli Lilly - Forsteo from EU market and Forteo from US market. No own formulation development was performed but followed the reference product formulation.

- Physicochemical and Biological Properties

At the early stage of product development an initial assessment of critical quality attributes (CQA) was performed to evaluate typical aspects of sterile liquid dosage forms that could potentially affect the product purity, strength and stability. These potential CQAs were derived from the QTPP and prior knowledge and were used to guide the product and process development.

Target values of quality attributes were determined based on the relevant Ph. Eur. chapters and regulatory guidelines

- Manufacturing Process Development

The commercial manufacturing process has been developed to produce single dose, single-use sterile prefilled syringes 20µg/ 80µl from drug substance via aseptic processing. As the drug substance is already provided pre-formulated and there are only few steps in drug product manufacturing, the process development could be more streamlined. A process was set up, where the manufacture of Bonosome r-DNA drug product involves only thawing of the drug substance, dilution with formulation buffer, sterile filtration, and filling into syringes

- Microbiological Attributes

Container Closure integrity testing is tested by leakage test. The drug product is tested for sterility and endotoxin as part of release and during stability studies.

- Compatibility

Bonosome r-DNA is a sterile liquid formulation for subcutaneous administration. No pre-use dilution or handling is applicable for drug product. The compatibility of the solution for injection with the immediate packaging material is monitored in the stability studies.

- **Manufacture of the drug product:**

  Description of manufacturing process and process controls along with manufacturers and responsibilities.

  Manufacturer:

  -The finished product manufacturing and batch release take place at Minapharm Pharmaceuticals and chemical industries.

  -The manufacturing process has been adequately validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of the intended quality in a reproducible manner.

-The manufacturing method consists of several stages, namely: dispensing, preparation of the concentrated formulation buffer, filtration and dilution of the concentrated formulation buffer, preparation of the bulk drug product solution with pre-filtration, final sterile filtration and filling of the siliconized sterilized syringes. The syringes are sealed with plunger stoppers and plunger rods.

- Control of critical steps and intermediates

The critical steps of the Bonosome r-DNA drug product manufacturing process along with the associated in-process tests and acceptance criteria are listed in the dossier.

- Process validation and / or evaluation

Bonosome r-DNA drug product (DP) manufacture is conducted in a qualified sterile manufacturing facility in accordance with current good manufacturing practices (cGMP). Aseptic processing is routinely challenged utilizing aseptic process simulations (APS). Results of the last successful media fill simulation (consecutive runs performed) are submitted**.**

- **Product specification:**

-The specifications proposed for release and stability testing of Bonosome r-DNA finished product comply with Ph. Eur. and USP.

-The specifications include appearance, general tests, tests for identity, tests for purity/product-related impurities, biological activity, quantity, tests for contaminants, and container closure integrity.

- Justification of the drug product specifications at the release and during stability studies are provided.

- All excipients used for Bonosome r-DNA drug product are compendial, non-novel excipients non-novel excipients such as (Sodium acetate, anhydrous and Acetic acid, glacial used as Buffer), (Mannitol as Osmotic diuretic), (Metacresol as Antimicrobial preservative), (Water for injection as Solvent) and (Nitrogen overlay as Inert gas).

-These components are controlled and tested to the standards appropriate for their intended use and function.

-no further impurities are introduced during the drug product manufacturing process.

- **Reference Standards or Materials.**

The reference standard is qualified to serve for release and stability assays for both drug substance and drug product.

- **Container closure system**

-Primary Packaging:

Bonosome r-DNA drug product is packaged in Glass barrel made from borosilicate glass type I, according to USP, EP, assembled with stainless steel needle with RNS (rigid needle shield) rubber plunger . And neutral plunger rod.

- **Stability of the drug product**

Based on available stability data, the proposed shelf-life of 2 year and storage conditions (store in a refrigerator, 2°C – 8°C, do not freeze).

Adventitious agents

Overall, the risk of contamination of bonosme with adventitious agents is considered low. The Applicant implemented multiple complementing measures to ensure product safety with regard to non-viral and viral adventitious agents. The measures include selection of materials, testing of cell

banks and process intermediates, testing of microbial attributes at release.These measures assure that Bonosome r-DNA does not contain adventitious agents.
The risk of potential contamination with viral adventitious agents in Bonosome rDNA is relatively low. Bonosome r-DNA is produced by fermentation in
Hansenula polymorpha therefore there is no significant risk of contamination
with viral adventitious agents.

Biosimilarity
-Analytical similarity of Bonosome was assessed in a comprehensive similarity exercise using the reference therapeutic product (Forteo). The approach and methodology of the analytical similarity assessment is sufficiently described and overall acceptable.
-Two main comparability assessments have been performed to bridge the produced drug substance materials from the different processes (Pre-clinical, Phase-I clinical, Commercial).
-To bridge the quality between the pre-clinical and phase-I clinical process, the first comparability was performed between the drug substances manufactured from each process has been demonstrated based on dedicated comparability assessment. After establishing thecommercial scale, further comparability assessment has been performed to bridge the quality of the clinical (phase-I) process to the commercial scale process.
-Batches included
batches of reference product (Forteo) and locally sourced reference product from the Egypt market (which has the same trade name and is considered identical to the licensed reference product) have been included in each comparability study to confirm a similar profile for the material manufactured from the different processes along the project development; (preclinical, phase-I clinical and commercial process).

-Comparability criteria.
A comprehensive analytical similarity evaluation was implemented using state of the art methodologies to assess similarity between Bonosome r-DNA and the reference medicinal products from Eli Lilly Forteo® (US-licensed reference product, available version in the Egyptian market) and Forsteo® (EU authorized reference product) and Forteo®. Similarity exercises involved assessing of various quality attributes including identity, primary structure, higher order structure, purity tests, product related modifications and biological characterisation (cell-based bioassays). Multiple batches of the reference therapeutic product were used to determine the variability ranges for the critical quality attributes. Bonosome r-DNA batches were assessed against the established corridor of reference therapeutic product. The assays performed in the clinical and commercial similarity assessment are as following:

Quality attribute	**Analytical method assessed**
Identity, content, purity	RP- HPLC
Purity	SDS-PAGE and Western blot
pI	Horizontal IEF
Identity, sequence verification, post-translational modifications	Sequence analysis by LC-ESI-MS
Identity	N-terminal Edman sequencing*

Aggregates	SEC-UV
Aggregates	AUC
Higher order structure	CD spectroscopy
Biological activity	In-vitro Cell-Based Bioassay (s)**

An orthogonal biological assay (USP monograph Bioidentity assay for Teriparatide Injection.) was performed in addition to the main cell-based bioassay. The additional cell-based bioassay was performed for a limited number of batches in the second comparability.

All the assays, comparability was demonstrated between the samples manufactured by the preclinical and phase-I clinical and commercial scale process, as well as to the reference products. All comparability results from the extended characterization with all performed analytical techniques are provided.

- Conclusion

The biosimilarity exercise is robust and there are no residual uncertainties arising from the quality comparison. Based on the obtained comparability results, comparability was demonstrated between the samples manufactured by the preclinical, clinical and commercial scale processes, as well as to the reference therapeutic product. From the overall biosimilarity exercise, it can be concluded that Bonosome r-DNA is similar to the reference medicinal product from Eli Lilly (Forteo®/Forsteo®).

3. Non –clinical aspect:

- **Pharmacology:**

In vitro

The applicant adopted a potency assay that uses a human cell line (Hek293) over-expressing the membrane-standing PTH1 receptor. The PTH1 receptor transduces PTH signaling resulting in cAMP formation. Cells respond with concentration dependent cAMP formation. Cells are lysed and cAMP concentration is measured with a commercial available cAMP Kit employing TR-FRET technology.
Potency screening of 10 different batches of Minapharm drug substance (final bulk), vs. 5 different batches of Minapharm drug product (Bonosome r-DNA syringes) vs. 8 different batches FORTEO® (reference product) were compared using a dedicated validated cell-based assay. Results for all samples were within the acceptance criteria for the assay. Statistical analysis showed no significant differences between neither Minapharm drug substance and drug product, Minapharm drug substance and FORTEO® nor Minapharm drug product and FORTEO®. Accordingly, it was concluded that Bonosome r-DNA and FORTEO® show identical interaction with the PTH1 receptor leading to the same potency results.

In vivo

Primary pharmacodynamics were addressed during a 4-week toxicity study of Bonosome r-DNA and its reference product FORTEO® by repeated daily subcutaneous administration to CD rats. To assess their bone architecture, the 60 rat femurs coming from sponsor experiment were analyzed by microcomputed tomography (micro-CT). It permits to determine bone mineral density (BMD) as well as histomorphometric parameters. Bonosome r-DNA as well as FORTEO® showed a pharmacodynamic effect after 4 treatment weeks, at trabecular level and to a lower extent on cortical thickness. Both compounds increased bone density at trabecular level. Both compounds were more effective on males than females. However the efficiency of both compounds was similar in males and females. In conclusion, Bonosome r-DNA increases bone volume and density with an identical efficiency as FORTEO®, whether male or female. Its effect remains on females even after 4 weeks of recovery time.

Pharmacokinetics:

As part of the development of Bonosome r-DNA, comparative single-dose pharmacokinetics as well as repeated dose pharmacokinetics versus FORTEO® were conducted in rats.

Single-dose pharmacokinetics were analysed in male CD rats at a dose level of 100 µg/kg b.w . The plasma levels of PTH were analysed using a validated ELISA assay. A non-compartmental model was applied. Highly similar mean C_{max} concentrations were noted for the animals treated once subcutaneously with Bonosome r-DNAor FORTEO® at a dose level of 100 µg/kg b.w. The $AUC_{0-\infty}$

values were highly comparable for Bonosome r-DNA and FORTEO®. The AUC0-∞ values were 17606 pg*h/mL plasma for r-PTH1-34 and 13182 pg*h/mL plasma for Forteo®

Repeated-dose pharmacokinetics were analysed in male and female CD® rats by daily repeated subcutaneous administration of the animals over 4 weeks at a dose level of 100 µg/kg b.w . The plasma levels of PTH were analyzed using a validated ELISA assay. A non-compartmental model was applied. Highly similar mean C_{max} concentrations were noted for the animals treated once subcutaneously with Bonosome r-DNA® or FORTEO® at a dose level of 100 µg/kg b.w. The $AUC_{0-\infty}$ values were highly comparable for Bonosome r-DNA® and FORTEO®.

In conclusion, Bonosome r-DNA® showed an identical pharmacokinetic profile compared to FORTEO® following subcutaneous administration.

- **Toxicology:**

A 4-week toxicity study with daily subcutaneous administration at different dose levels (10, 30 and 100 mcg PTH/kg b.w.) including a 4-week recovery period for Bonosome r-DNA compared to the reference product FORTEO® was conducted in CD rats. The aim of the study was to obtain information on the toxicity and to assess the reversibility of any effect after a recovery period. No

deaths were reported .

No noteworthy differences were noted between the haematological parameters, organ weights, histopathological findings, and bone parameters of the animals treated with Bonosome r-DNA and the animals treated with FORTEO® during a 4-week repeated dose toxicology study in rats.

The clinical relevance of anti-teriparatide antibodies is considered negligible. There is no evidence that these antibodies neutralize the biological activity of teriparatide or produce any adverse clinical outcomes. Nevertheless, the applicant addressed the formation of anti-teriparatide antibodies during a 4-week toxicity study with daily subcutaneous administration at different dose levels (10, 30 and 100 mcg PTH/kg b.w.) including a 4-week recovery period using a self-developed specific ELISA method. The applicant could not detect any formation of anti-drug-antibodies for animals treated with Bonosome r-DNA or FORTEO®.

No new genotoxicity, carcinogenicity norreproductive and developmental toxicity studies were conducted with teriparatide. In compliance with ICH guidance (EMEA/CHMP/BMWP/42832), this is acceptable.

Local tolerance was assessed as part of the 4-week repeated dose toxicity study of Bonosome r-DNA and FORTEO®. Findings similar in appearance and incidence were observed across all groups. The findings were therefore considered to be related to the experimental procedure and/or the vehicle and not to teriparatide treatment.

- **Conclusion on non-clinical aspects**

The provided non-clinical comparability testing strategy is regarded as sufficient in the context of a biosimilar development. Corresponding national and international regulatory guidelines were taken into consideration. Comparative in-vivo pharmacodynamic, pharmacokinetic and toxicology data, as well as in-vitro pharmacological assessment, supports the totality-of-the-evidence that demonstrates the similarity between Bonosome r-DNA® and the reference product Forteo®.

4. Clinical aspect

- **Clinical Pharmacology conclusion:**

The clinical development program to show biosimilarity between Bonosome r-DNA® (teriparatide 20 µg/80 µL solution for injection) and Forsteo® consists of a single comparative pharmacokinetic (PK) study in 54 healthy women

- Tabular overview of clinical studies

Table 1

Location of study report/CTD code	Type of study	Study identifier (study/report number)	Objectives of the study	Study design and type of control	Test products; Dosage regimen; Route of admin.	No. of subjects	Healthy subjects or diagnosis of patients	Duration of treatment	Study status; type of report
Module 5.3.2.1	Phase 1 Comparative PK/PD; safety/local tolerance Equivalence study	MP-BO1-01 EudraCT No: 2020-002984-7	Primary: Demonstrate PK equivalence between Bonosome® and FORSTEO® Secondary: Evaluate PD and safety/local tolerance of Bonosome® compared to FORSTEO®	Randomised, double-blind, single-center, single-dose, fixed-dose, 2-way-cross-over, comparative PK study Active controlled (vs EU sourced teriparatide reference product FORSTEO®	Teriparatide 20 ug/ul s.c. Treatm. A Bonosome (Batch No. 0914) 20 ug/80ul Single dose; s.c. inject. Treatm. B FORSTEO (Batch No D233847C) 20 ug/80 ul Single dose; s.c. inject.	Enrolled: N= 54 Completed: N= 54	Healthy adult female pre-monopausal subjects	Single-dose 2 periods of appr. 12 hours each; washout 4-7 days between both doses	Completed; final CSR

Pharmacokinetics

The comparison of the PK profiles of Bonosome r-DNA and FORSTEO®was the primary objective of study MP-BO1-01, which is described below.

- *Study Design*

Study MP-BO1-01 was a single center, phase I, double-blind (observer-blinded), randomized, single dose, fixed dose, 2-period, 2-way cross-over comparative pharmacokinetic study of teriparatide in healthy female adult volunteers planned to compare the PK of Bonosome r-DNA (biosimilar teriparatide) with that of the reference medicinal product Forsteo (EU-sourced) in 54 healthy adult female subjects. Subjects were randomised to treatment sequences AB or BA, where treatment A was the single-dose administration of Bonosome r-DNA and treatment B was the single-dose administration of FORSTEO®.

- *Study participants*

Of the 54 healthy adult female subjects entering the study and being randomised to either of the two treatment arms, 54 completed both periods and 54 were included in the final PK-analysis set.The mean age for all subjects was 36.7 years (range 20-54 years), the mean weight was 66.3 kg (range 49.0-95.0

kg), the mean height was 166.5 cm (range 151– 186 cm), and the mean body mass index (BMI) was 23.9 kg/m2 (range 19– 29 kg/m2).

- *Treatments*

- Treatment A (test): A single 20 µg/80 µL SC injection of Bonosome r-DNA (Minapharm Pharmaceuticals)

Treatment B (reference): A single 20 µg/80 µL sc injection of FORSTEO® (Lilly France S.A.S.) Subjects received the subcutaneous injection in a semi-reclined or supine position, into the right side of the abdomen in period 1, into the left side of the abdomen in period 2.

- *Results*

Primary PK endpoints

Table 2: Primary pharmacokinetic parameters of teriparatide in plasma – pharmacokinetic analysis set

Pharmacokinetic parameter [unit]		Treatment A – Test Bonosome r-DNA N=54	Treatment B – Reference FORSTEO® N=54
$AUC_{0-tlast}$ [pg/mL*h]	n	54	54
	Geometric mean	54.98	52.63
	Geometric CV	46.44	41.35
	Min-Max	11.3-137.9	26.8-127.5
C_{max} [pg/mL]	n	54	54
	Geometric mean	67.19	69.69
	Geometric CV	40.05	35.96
	Min-Max	27.8-142.4	34.0-154.1

n: number of subjects, CV: coefficient of variation, Min: minimum, Max: maximum

Secondary PK endpoints

Table 3: Secondary pharmacokinetic parameters of teriparatide in plasma – pharmacokinetic analysis set

Pharmacokinetic parameter [unit]		Treatment A – Test Bonosome r-DNA N=54	Treatment B – Reference FORSTEO® N=54
t_{max} [h]	n	54	54
	Geometric mean	0.25	0.25
	Geometric CV	48.24	46.26
	Median	0.17	0.18
	Min-Max	0.2-0.8	0.2-0.6
$t_{1/2}$ [h]	n	54	54
	Geometric mean	0.53	0.42
	Geometric CV	46.46	39.65
	Min-Max	0.3-2.2	0.1-1.0

n: number of subjects, CV: coefficient of variation, Min: minimum, Max: maximum

Other PK endpoints

Table 4: Other pharmacokinetic parameters of teriparatide in plasma – pharmacokinetic analysis set

Pharmacokinetic parameter [unit]		Treatment A – Test Bonosome r-DNA N=54	Treatment B – Reference FORSTEO® N=54
$AUC_{0-\infty}$ [pg/mL*h]	n	54	54
	Geometric mean	68.67	62.17
	Geometric CV	36.25	39.07
	Min-Max	25.0-143.3	32.3-138.8
$AUC_{ext\ [\%]}$	n	54	54
	Geometric mean	15.85	13.68
	Geometric CV	64.10	47.77
	Min-Max	3.8-62.2	4.8-30.7
t_{last} [h]	n	54	54
	Geometric mean	1.51	1.41
	Geometric CV	22.41	20.43
	Min-Max	0.7-3.0	1.0-3.0

n: number of subjects, CV: coefficient of variation, Min: minimum, Max: maximum

Table 5: Summary of statistical analysis of pharmacokinetic parameters of teriparatide – pharmacokinetic analysis set

			Least square means		Least square mean ratio (Treatment A/B - test/reference)		
Pharmacokinetic parameter	N	n	Treatment A Test Bonosome r-DNA	Treatment B Reference FORSTEO®	Point estimate [%]	90% confidence intervals [%]	Intra-subject coefficient of variation [%]
$AUC_{0-tlast}$ [pg/mL*h]	54	54	54.98	52.63	104.46	97.33, 112.11	22.20
C_{max} [pg/mL]	54	54	67.19	69.69	96.41	90.63, 102.56	19.35
$AUC_{0-\infty}$[pg/mL*h]	54	54	68.67	62.17	110.46	103.56, 117.82	20.21

N = Number of evaluable subjects, n = number of evaluable subjects included in the model
Results are based on an analysis of variance model with sequence, period, treatment and subject nested within sequence as fixed effects.

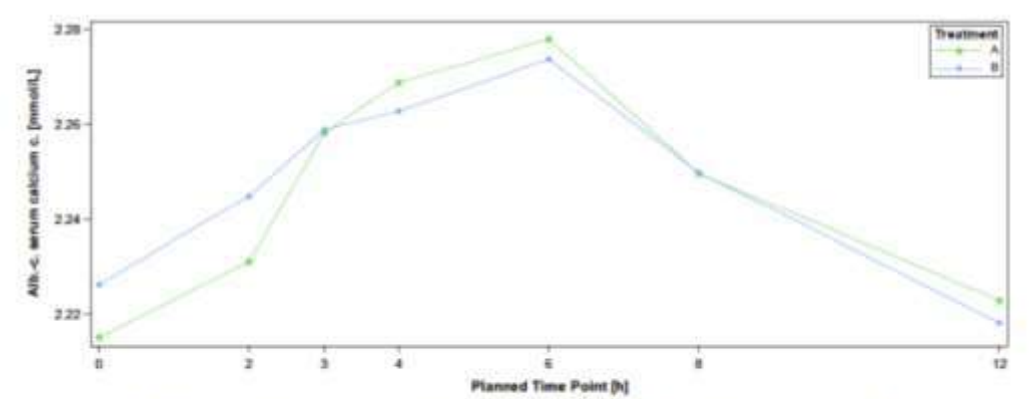
Conclusion on pharmacokinetics

PK biosimilarity of Bonosome r-DNA to FORSTEO® was demonstrated after single dose s.c. administration of 20 µg teriparatide. The 90% CIs for the primary PK endpoints $AUC_{0-tlast}$ [97.33%, 112.11%] and C_{max} [90.63%, 102.56%] were within the biosimilarity ranges of 80.00% to 125.00%.

Pharmacodynamics

For the information of the PD properties of teriparatide, the applicant provided supportive data from the PK study, where increases in serum calcium upon teriparatide application were measured and evaluated as a PD parameter. From the reference product FORSTEO®, teriparatide is known to cause transient increases in calcium after each dose. This is observed in healthy volunteers and patients and is believed to be due to increased intestinal absorption and increased tubular reabsorption of calcium in response to teriparatide. Comparative albumin-corrected serum calcium and phosphate concentration-time curves for Bonosome r-DNA and FORSTEO® are provided. The analysis includes statistical analysis on the PD parameters $AUC_{0\text{-}tlast}$, and C_{max}. 90% confidence intervals are provided.

Figure 1: Geometric mean albumin corrected serum calcium concentrations-time curve, linear scale – pharmacodynamic analysis set



The curve includes only those time points where at least 50% of values are equal or above LLOQ.
Treatment A = Test, 20 µg teriparatide as s.c. injection (Bonosome r-DNA)
Treatment B = Reference, 20 µg teriparatide as s.c. injection (FORSTEO®)

Table 6: Pharmacodynamic parameters of albumin-corrected serum calcium concentration – pharmacodynamic analysis set

Pharmacodynamic parameter		Treatment A – Test Bonosome r-DNA N=54	Treatment B – Reference FORSTEO® N=54
$AUC_{0\text{-}t_{last}}$ [h*mmol/L]	n	54	54
	Geometric mean	27.000	26.993
	Geometric CV	2.650	2.200
	Min-Max	25.47-28.70	25.71-28.34
C_{max} [mmol/L]	n	54	54
	Geometric mean	2.297	2.296
	Geometric CV	2.630	2.250
	Min-Max	2.16-2.42	2.17-2.41
t_{max} [h]	n	54	54
	Geometric mean	4.494	Not defined
	Geometric CV	45.158	Not defined
	Mean (SD)	4.912 (2.138)	4.931 (2.429)
	Median	4.010	6.000
	Min-Max	2.00-12.00	0.00-12.02

n: number of subjects, CV: coefficient of variation, Min: minimum, Max: maximum, SD: standard deviation

Table 7: Pharmacodynamic parameters of serum phosphate concentration – pharmacodynamic analysis set

Pharmacodynamic parameter		Treatment A – Test Bonosome r-DNA N=54	Treatment B – Reference FORSTEO® N=54
$AUC_{0\text{-}t_{last}}$ [h*mmol/L]	n	54	54
	Geometric mean	13.251	13.272
	Geometric CV	10.106	10.811
	Min-Max	9.77-16.70	9.96-16.32
C_{max} [mmol/L]	n	54	54
	Geometric mean	1.268	1.270
	Geometric CV	10.569	11.983
	Min-Max	0.98-1.64	0.93-1.56
t_{max} [h]	n	54	54
	Geometric mean	Not defined	Not defined
	Geometric CV	Not defined	Not defined
	Mean (SD)	9.785 (4.254)	9.931 (3.921)
	Median	12.000	12.000
	Min-Max	0.00-12.25	0.00-12.12

n: number of subjects, CV: coefficient of variation, Min: minimum, Max: maximum, SD: standard deviation

Table 8: Summary of statistical analysis of secondary pharmacodynamic parameters (albumin-corrected serum calcium)– pharmacokinetic analysis set

Pharmacodynamic parameter	N	n	Least square means		Least square mean ratio (Treatment A/B - test/reference)		
			Treatment A Test Bonosome r-DNA	Treatment B Reference FORSTEO®	Point estimate [%]	90% Confidence intervals [%]	Intra-subject coefficient of variation [%]
$AUC_{0\text{-}tlast}$ [h*mmol/L]	54	54	27.00	26.99	100.03	99.68, 100.37	1.07
C_{max} [mmol/L]	54	54	2.30	2.30	100.07	99.68, 100.46	1.22

LS: least square, N: number of evaluable subjects; n: number if evaluable subjects included in the model

Table 9: Summary of statistical analysis of other pharmacodynamic parameters (serum phosphate) pharmacokinetic analysis set

Pharmacodynamic parameter	N	n	Least square means		Least square mean ratio (Treatment A/B - test/reference)		
			Treatment A Test Bonosome r-DNA	Treatment B Reference FORSTEO®	Point estimate [%]	90% confidence intervals [%]	Intra-subject coefficient of variation [%]
$AUC_{0\text{-}tlast}$ [h*mmol/L]	54	54	13.25	13.27	99.84	98.04, 101.67	5.65
C_{max} [mmol/L]	54	54	1.27	1.27	99.83	97.73, 101.98	6.61

LS: least square, N: number of evaluable subjects; n: number if evaluable subjects included in the model

PD similarity of Bonosome r-DNA to FORSTEO® was demonstrated after single dose s.c. administration of 20 µg teriparatide. The 90% CIs for the secondary PD endpoints $AUC_{0\text{-}tlast}$ [99.68%, 100.37%] and for C_{max} [99.68%, 100.46%] for albumin-corrected serum calcium concentrations were within the similarity range of 80.00% to 125.00. The 90% CIs for the other PD endpoints $AUC_{0\text{-}tlast}$ [98.04%, 101.67%] and C_{max} [97.73%, 101.98%] for serum phosphate concentrations were within the similarity ranges of 80.00% to 125.00%.

Clinical efficacy conclusion

No dedicated clinical efficacy study has been performed, which is acceptable for teriparatide in principle.

Several PD parameters (albumin-corrected serum calcium and phosphate concentrations) were analysed comparatively as surrogates for efficacy (see also clinical pharmacology conclusion).

Clinical safety conclusion

Overall, single doses of 20 µg teriparatide as SC administration, were safe and well tolerated in clinical study MP-BO1-01.

The observed TEAEs were mainly mild, transient, and manageable and the AE profile was in line with previous experience with teriparatide. No relevant difference was observed between Bonosome r-DNA and the reference product FORSTEO®. No safety-relevant effects on safety laboratory parameters, vital signs, or ECG parameters were observed. Skin reactions observed at the administration side (erythema/redness, pain and itching) occurred after both, the test and reference treatment. All skin reactions were mild and only of short duration. The investigator assessed the overall tolerability at final check as good for all subjects.
No new or changed risks associated with the use of teriparatide were identified.

Clinical immunogenicity conclusion
Anti-drug antibodies were addressed in plasma samples collected in the MP-BO1-01 PK/PD study using a specific, validated ELISA assay. In none of the tested samples, the presence of treatment-positive ADAs was confirmed. Hence, no evidence for immunogenicity was found.

5. Benefit/risk conclusion

For a biosimilar, the benefit-risk balance is derived from the reference product, provided the totality of evidence collected from the physicochemical and biological characterisation and the non-clinical and clinical data package supports the comparability of the two products, which EDA considered to be the case.
The overall benefit/risk balance of Bonosome r-DNA 20 µg/80 µL solution for injection is positive.
Based on 2 inspection visits to the Minapharm factory & to the clinical site in Germany and based on the review of data on quality, safety and efficacy, the EDA considers by consensus that the risk-benefit balance of Bonosome r-DNA is favourable in the following indication:
Bonosome r-DNA is indicated in adults.
Treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture.
In postmenopausal women, a significant reduction in the incidence of vertebral and non-vertebral fractures but not hip fractures has been demonstrated.
Treatment of osteoporosis associated with sustained systemic glucocorticoid therapy in women and men at increased risk for fracture.