13 November 2025
EMA/37498/2026
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Osqay

International non-proprietary name/Common name: Denosumab

Procedure No. EMEA/H/C/006492/0000

Note

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

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List of abbreviations

ADA	Anti-Drug Antibody
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Concentration Time Curve
AUC0-∞	Area under the concentration versus time curve from time zero to infinity
AUC0-1M	Area Under the Concentration Time Curve From Time 0 To Month 1
AUC0-28 days	Partial Area Under The Drug Concentration-Time Curve From Time 0 (Pre Dose) To Day 28
AUC0-3M	Area Under The Concentration Time Curve From Time 0 To Month 3
AUC0-6M	Area Under The Concentration Time Curve From Time 0 To Month 6
AUC0-inf	Area Under the Drug Concentration-Time Curve From Time 0 To Infinity
AUC0-t	Area Under the Drug Concentration-Time Curve From Day 0 To Day 270
AUC6M-12M	Area Under the Concentration Time Curve From Month 6 To Month12
AUEC	Area Under Effect Curve
AUEC0-t	Area under the effect curve from time zero to the last measurable concentration
BIA	Biosimilar Initial Advisory
BMD	Bone Mineral Density
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CCMV	Completer case missing value
CFB	Change from Baseline
CHMP	The Committee for Medicinal Products for Human Use
CI	Confidence Interval
Cmax	Maximum measured concentration
COVID-19	Coronavirus Disease-2019
CRF/eCRF	Case Report Form/Electronic Case Report Form
CRO	Contract Research Organisation
CSR	Clinical Study Report
Ctrough 12M	Ctrough Concentration on Month 12
Ctrough 6M	Ctrough concentration on month 6
CTX	Serum cross-linked C-telopeptide of type I collagen
CTX1	Serum C-Telopeptide of Type I Collagen
CV	Coefficient of variation

DP	Drug Product
DS	Drug Substance
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
ECLIA	Electrochemiluminescence immunoassay
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked immune sorbent assay
EMA	European Medicines Agency
Emax	Maximum % reduction from baseline
ENZ215	Proposed Biosimilar to Prolia®
EOS	End of study
EU	European Union
US FDA	United States Food and Drug Administration
FREEDOM	Fracture reduction evaluation of denosumab in osteoporosis every six months
GCP	Good Clinical Practice
geoCV	Geometric Coefficient of Variation
GMR	Geometric Mean Ratio
h	Hour
H	Hour
HPC	High Positive Control
HQC	High Quality Control
HRP	Horseradish Peroxidase
ICE	Intercurrent event
ICH	The International Council for Harmonisation Of Technical Requirements For Pharmaceuticals For Human Use
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
IP	Investigational Product
IQC	Instrument Quality Control
IRB	Institutional Review Board
ISR	Incurred Sample Reanalysis
ITT	Intent-To-Treat
IU	International Units
LLOQ	Lower Limit of Quantification
LPC	Low Positive Control
LS Mean	Least Square Mean
LS-BMD	Bone Mineral Density At Lumbar Spine
LSM	Least-squares means

MCH	Mean Cell Haemoglobin
MCV	Mean Corpuscular Volume
mITT	Modified Intent-To-Treat
MMRM	Mixed Model For Repeated Measures
MNAR	Missing not at random
MRD	Minimum Required Dilution
NAb	Neutralizing Antibodies
NC	Negative Control
ng/mL	Nanogram per milliliter
OD	Optical Density
ONJ	Osteonecrosis of Jaw
ORR	Objective Response Rate
P1NP	Procollagen Type 1 N-Terminal Propeptide
PD	Pharmacodynamic(s)
PI	Package Insert
PK	Pharmacokinetic(s)
PKAS	Pharmacokinetic analysis set
PMO	Post-menopausal Osteoporosis
PP	Per Protocol
PT	Preferred Term
QC	Quality Control
QTL	Quality Tolerance Limits
RANKL	Receptor Activator Of Nuclear Factor Kappa-B Ligand
RBC	Red Blood Cells
RLU	Relative Light Units
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SC	Subcutaneous
sCTX	Serum C-Telopeptide Of Type 1 Collagen
SD	Standard Deviation
SmPC	Summary Of Product Characteristics
SOC	System Organ Class
sP1NP	Serum Procollagen Type 1 N-Terminal Propeptide
SUSAR	Suspected Unexpected Serious Adverse Reaction
t1/2	Terminal half-life
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
Tmax	Time of the maximum measured concentration
TMB	3,3’,5,5’-Tetramethylbenzidine
ULOQ	Upper Limit of Quantitation

US	United States
USFDA	United States Food and Drug Administration
USPI	United States Prescribing Information
WHO	World Health Organization
WMA	World Medical Association
XCAL	Inter-Scanner Cross-Calibration

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Theramex Ireland Limited submitted on 18 November 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Osqay, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

> Treatment of osteoporosis in postmenopausal women and in men at increased risk of fractures. In postmenopausal women denosumab significantly reduces the risk of vertebral, non-vertebral and hip fractures.
>
> Treatment of bone loss associated with hormone ablation in men with prostate cancer at increased risk of fractures (see section 5.1). In men with prostate cancer receiving hormone ablation, denosumab significantly reduces the risk of vertebral fractures.
>
> Treatment of bone loss associated with long-term systemic glucocorticoid therapy in adult patients at increased risk of fracture (see section 5.1).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

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

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

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 8 years in the EEA:

- Product name, strength, pharmaceutical form: Prolia 60 mg solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.; Minervum 7061; 4817 ZK Breda; The Netherlands
- Date of authorisation: 26-05-2010
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/10/618

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

- Product name, strength, pharmaceutical form: Prolia 60 mg solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.; Minervum 7061; 4817 ZK Breda; The Netherlands
- Date of authorisation: 26-05-2010
- Marketing authorisation granted by:
  - Union

- Marketing authorisation number: EU/1/10/618

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: Prolia 60 mg solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.; Minervum 7061; 4817 ZK Breda; The Netherlands
- Date of authorisation: 26-05-2010
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/10/618

1.3. Information on Paediatric requirements

Not applicable

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Scientific advice

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

Date	Reference	SAWP co-ordinators
15 October 2020	EMEA/H/SA/4580/1/2020/III	Elina Rönnemaa, Juha Kolehmainen
16 September 2021	EMA/SA/0000060480	Elina Rönnemaa, Mogens Westergaard

EMEA/H/SA/4580/1/2020/III

The Applicant received Scientific advice on the development of denosumab biosimilar (ENZ215) for the treatment in the same indications as the reference product Prolia/Xgeva from the CHMP on 15 October 2020 (EMEA/H/SA/4580/1/2020/III). The Scientific advice pertained to the following quality, non-clinical, and clinical aspects:

- The proposed approach to criticality assessment of analytical similarity; the physicochemical and functional similarity approach; the strategy of establishing structure function relationship of quality attributes; the approach to determining release parameters for drug substance and drug product; the

proposed stability programme; the choice of tests for batch release testing; the strategy to establish nternal reference standard for ENZ215; the proposed release assays; the approach and studies for studies for integrated continuous downstream processing; the strategy for assessment of product contact materials, impact of freeze thaw, leachables, prefilled syringe siliconization; the approach to assess comparability between early and late process product; the strategy for master cell bank and working cell bank characterization for GMP banks.

- The adequacy of non-clinical evidence of similarity between ENZ215 and the reference products.
- The design of a randomized, open label, two-stage clinical study to establish pharmacokinetic comparability between ENZ215 and the reference product, including the proposed population of healthy volunteers, the proposed sub-therapeutic dose, the primary endpoints and secondary endpoints, the statistical assumptions including sample size and analyses of the PK endpoints.
- The design of a randomized, double-blind, parallel-group, active-controlled study to compare the efficacy, safety, and immunogenicity of ENZ215 and Prolia, including the proposed population, the primary endpoints and secondary endpoints, the proposed approach to immunogenicity assessment, the statistical assumptions including sample size and analyses of the efficacy endpoints.
- The approach to the use of clinical data generated outside of the EU with US-sourced reference product for a marketing authorization application in the EU and the approach to the use of US-sourced reference product in clinical studies; the overall strategy for immunogenicity assessment.

EMA/SA/0000060480

The Applicant received Scientific advice on the development of denosumab biosimilar (ENZ215) for the treatment in the same indications as the reference product Prolia/Xgeva from the CHMP on 16 September 2021 (EMA/SA/0000060480). The Scientific advice pertained to the following clinical aspects:

- The design of a randomized, double-blind, single-dose study in healthy volunteers to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity of ENZ215 with the reference medicinal product including primary and secondary endpoints, sampling time points, study population, sample size, injection site, geography of patient enrolment.
- The design of a randomized, double-blind clinical study in postmenopausal women with osteoporosis to compare the efficacy, safety, pharmacodynamics, pharmacokinetics and immunogenicity of ENZ215 with the reference product including pharmacokinetic assessment in a subset of patients, primary and secondary endpoints, safety endpoints, exclusion criteria, sample size, efficacy analysis data set, geography of patient enrolment.

1.6. Steps taken for the assessment of the product

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

Rapporteur: Christian Gartner Co-Rapporteur: Kristina Nadrah

The application was received by the EMA on	18 November 2024
The procedure started on	27 December 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 March 2025

The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	31 March 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	31 March 2025
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 April 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	29 July 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	25 August 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	4 September 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	11 September 2025
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	18 September 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	13 October 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	29 October 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	29 October 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Osqay on	13 November 2025

2. Scientific discussion

2.1. About the product

Osqay was developed as a biosimilar product to Prolia (INN: denosumab), marketed by Amgen and was developed with the same strength and presentation:

- Prolia: 60 mg/mL PFS

The Applicant is claiming all the indications approved for the reference product.

Prolia indications:

- Treatment of osteoporosis in postmenopausal women and in men at increased risk of fractures. In postmenopausal women Prolia significantly reduces the risk of vertebral, non-vertebral and hip fractures
- Treatment of bone loss associated with hormone ablation in men with prostate cancer at increased risk of fractures. In men with prostate cancer receiving hormone ablation, Prolia significantly reduces the risk of vertebral fractures
- Treatment of bone loss associated with long-term systemic glucocorticoid therapy in adult patients at increased risk of fracture

2.2. Type of Application and aspects on development

Development program: Two clinical studies have been conducted as follows:

- Clinical Phase 1 study (ALK22/ENZ215-DEN1): A randomized, double-blind, three-arm, parallel-group, single-dose study to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity of Denosumab (ENZ215, EU-sourced Prolia, and US-sourced Prolia) in healthy adult male volunteers
- Clinical Phase 3 study (ALK22/ENZ215-DEN2): A Phase 3, Randomised, Double-blind, Parallel-group, Active-controlled Study to Compare the Efficacy, Safety, Pharmacodynamics, Pharmacokinetics and Immunogenicity of Enzene Denosumab (ENZ215) and Prolia in Postmenopausal Women with Osteoporosis.

Pediatrics: Since the application is submitted as a biosimilar application under article 10 (4) of Directive 2001/83/EC, no information relating to paediatrics was provided by the Applicant.

Scientific advice: The Applicant obtained scientific advice on 15 Oct 2020 (EMA/CHMP/SAWP/531236/2020) including a clarification letter (29 Jan 2021; EMEA/H/SA/4580/1/2020/III), and a follow up advice on 16 Sep 2021 (EMA/SA/0000060480). All aspects that were discussed critically during these advice procedures and are deviating from the final study designs will be discussed in the respective methods or result section. The Applicant followed in main aspects the advice provided by the CHMP.

2.3. Quality aspects

2.3.1. Introduction

Osqay contains denosumab (INN) as the active substance, also referred to as ENZ215, which is a human monoclonal IgG2 monoclonal antibody produced in a Chinese hamster ovary (CHO) cell line by recombinant DNA technology.

Other ingredients are glacial acetic acid, sodium hydroxide, sorbitol, polysorbate 20 and water for injections (WFI).

Osqay is presented as a solution for subcutaneous injection in a single use pre-filled syringe (PFS). Each PFS contains 60 mg of denosumab in 1 mL of solution.

Osqay has been developed as a biosimilar to the reference medicinal product (RMP) Prolia (EMEA/H/C/001120).

2.3.2. Active Substance

2.3.2.1. General Information

Denosumab (ENZ215) is a human monoclonal IgG type 2 monoclonal antibody that has an approximate molecular weight of 147 kDa. It is produced in genetically engineered mammalian CHO cells. It consists of 2 heavy chains (HC) and 2 light chains (LC) of the kappa subclass. Denosumab contains 36 total cysteine residues, which are involved in both intrachain and interchain disulphide bonds. Each heavy chain contains an N-linked glycan at the consensus glycosylation site at Asparagine 298.

Denosumab targets and binds with high affinity and specificity to RANKL, preventing activation of its receptor, RANK, on the surface of osteoclast precursors and osteoclasts.

2.3.2.2. Manufacture, process controls and characterisation

Manufacturing process

The active substance (AS) is manufactured at Enzene Biosciences Ltd., Plot No. A/22 A/1/2, MIDC Chakan Industrial Area Phase II, Khalumbre Village, Khed Taluka, Pune 410501, India. The competent authority of the Netherlands performed a pre-authorisation GMP inspection of this site and issued a GMP certificate. All sites involved in manufacture and control of the active substance operate in accordance with EU GMP.

The active substance is expressed in a CHO cell line and produced from a single vial of the working cell bank (WCB) expansion through multiple stages, followed by harvest. The harvest is purified by series of purification steps including chromatography, virus inactivation and removal.

The Applicant provided a detailed description of the manufacturing process steps that is accompanied by flow charts and tables listing process parameters and in-process controls (IPCs) with their classification and acceptable ranges/acceptance criteria/action limits.

In conclusion, the Applicant provided a detailed description of the manufacturing process and controls that is in line with regulatory expectations.

Control of critical steps and intermediates

The process controls during the manufacturing process of ENZ215 are categorised in OPs and IPCs. For OPs operating and acceptable ranges/limits/set-points are defined.

The manufacturing process is considered under control and the OPs and IPCs and their respective ranges/limits are adequate.

Control of materials

Raw materials used for upstream cell culture and downstream purification are listed together with the stage and their intended use. Certificates of analysis of suppliers are provided and testing complies with Ph. Eur. and

USP. Acceptable in-house specifications are provided for the non-compendial raw materials. The information provided is sufficient.

The Chinese Hamster Ovary (GCHO) parental cell line was transduced with Denosumab gene sequences.

A two-tiered cell bank system was developed for the manufacturing of ENZ215 active substance. A flow chart of MCB and WCB preparation is presented. MCB, WCB and end-of-production cell bank (EPCB) were characterised according to ICH Q5D. The characterisation tests included culture purity, identity, and stability. This is endorsed. Periodic stability testing and a protocol for future WCB preparation and characterisation are in place. This is supported. Absence of adventitious agents was confirmed.

Process validation

The manufacturing process was validated.

Three consecutive batches were manufactured. Overall, critical and non-critical process parameters were consistent for the three ENZ215 active substance batches during the upstream manufacturing process validation, as the process parameters were within acceptance criteria. Process validation of the downstream manufacturing process consists of buffer and solution preparation and downstream manufacturing process. Critical and non-critical process parameters and in-process tests are provided for each downstream purification step. All the deviations were investigated and adequately described and measures were taken.

Hold times

Overall, the presented hold-times at scale are considered meaningful and are acceptable.

Overall, the presented process validation data demonstrate that the intended commercial manufacturing process performs consistently and delivers active substance complying with the release specifications under commercial operating conditions.

Manufacturing process development

Process characterisation

Development of the active substance manufacturing process started with definition of the quality target product profile (QTPP), which describes the quality attributes of the denosumab molecule.

The acceptance ranges of operating parameters are sufficiently described. Parameter classification is deemed meaningful.

Comparability

The process for manufacturing of ENZ215 went through several changes to mature into the final intended commercial process of ENZ215 active substance. The comparability exercise to support various changes was presented. Extensive analysis considering PPQ was also done.

Characterisation

Elucidation of structure

ENZ215 active substance was characterised using state-of-the-art methodologies to evaluate the primary and higher order structures, purity, concentration, glycosylation and functional activity. Characterisation was done on active substance PPQ batches.

Impurities

Spiking studies using the qualified scale-down models were used to evaluate the process capabilities to reduce process- and product-related impurities to acceptable levels.

The impurity spiking and clearance studies were performed to demonstrate the capability of the chromatography steps.

An acceptable risk assessment for potential nitrosamine impurities has been provided where no risk was identified for ENZ215, but it is referred to 3.2.P. for further details.

The provided information regarding characterisation of the active substance is in line with the Guideline on development, production, characterisation and specification for monoclonal antibodies and related products EMA/CHMP/BWP/532517/2008 and sufficient.

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

Specification and justification of specification

The proposed release specification for ENZ215 active substance includes compendial tests for physical appearance (colour, clarity of solution), pH and osmolality. Quantification (OD280), identity (*in vitro* bioassay and SE-HPLC) and purity (CE-HPLC, SE-HPLC) testing is also listed. Safety testing by HCP content testing, residual protein A, HCD, Bacterial endotoxins (BET) (LAL) and bioburden is done. Potency testing is done via an *in vitro* bioassay for denosumab.

The stability specification test method acceptance criteria are set as tight as for release specifications and comprise the same applied methods except testing for identity and safety. This is deemed acceptable. Ph. Eur. references for the compendial methods and identifiers for in-house analytical methods.

An orthogonal method, CE-SDS as a complementary approach to the CE-HPLC method, has been introduced in active substance and finished product specifications. CE-SDS offers enhanced sensitivity for detecting size variants, including LMW species.

The proposed specification limits are agreeable.

Several of the described analytical methods are also used at finished product release.

The acceptance criteria and the overall control strategy have been established based on product specific knowledge and release/stability in accordance with ICH Q6B.

Setting of specifications is acceptable.

Analytical procedures and validation of analytical procedures

The general and microbial attributes are tested according to the respective Ph. Eur. monographs; all other attributes are tested using in-house analytical methods. The analytical methods are adequate for their intended purpose.

For all analytical methods validation summaries and the detailed validation reports have been submitted (reports are filed in attachments). The validation results demonstrate suitability of the analytical procedures for their intended use. The relevant parameters have been assessed in accordance with ICH Q2(R1). Robustness of the methods has been satisfactorily demonstrated for a set of relevant variables. Suitability of the microbiological compendial tests (i.e. bioburden and endotoxin) was adequately verified.

Batch Analyses

Batch analyses data are presented for batches each manufactured for phase 1 clinical trial scale or at the intended commercial scale process, including phase 3 trial batches, stability batches, RS batches and PPQ batches. All results comply with the specifications valid at time of testing and with the proposed commercial specifications (if applicable). In summary, the presented results demonstrate that the manufacturing process reliably delivers active substance with consistent quality.

Reference standard

The history of the developmental and primary internal reference standards (dIRS and pIRS) used throughout development of ENZ215 is adequately described. The reference standards were manufactured from ENZ215 active substance batches representing the respective development stage.

A standard two-tiered approach using primary and working reference standards is described and implemented.

The stability program in place for the internal reference standards has been sufficiently described and is provided for pIRS and dIRS.

The analytical programme for future pIRS re-qualification is acceptable. Results of the first re-qualification support further use of the current pIRS. Re-qualification of wIRS is adequately described in sufficient detail.

The establishment and the (re-)qualification of the reference standards are in the main well described.

The Applicant presented actual strategies to encounter possible shifts and drifts in reference standard re-qualification.

Container closure system

The container closure system used to store ENZ215 active substance. bags are sterile, pre-assembled and single-use containers.

Technical drawing as well as a representative Certificate of Analyses are provided. According to the Applicant all product contact materials are manufactured without the use of animal-derived components. Specifications are further included.

A scoring system was used to understand the risk associated with leachable. Based on results of this risk assessment score for the ENZ215 active substance container closure system leachable study are performed.

An extractable study was submitted.

An evaluation of potential leachable components of the active substance container has been performed.

A calculating the worst-case patient exposure to each compound and then comparing this exposure to permitted daily exposure (PDE) levels based on toxicological assessment according to ICH Q3D were performed.

In summary the Applicant’s conclusion that there is no safety concern for using the bag for active substance storage can be agreed.

2.3.2.4. Stability

The shelf life of the active substance has been suitably demonstrated.

The stability data provided by the Applicant included Phase 1 clinical campaign batches at real time (-20°C ± 5°C) and accelerated (5 ºC ± 3 ºC) conditions. Furthermore, batches from the Phase 3 clinical campaign were evaluated under the aforementioned stability conditions.

The batches were tested against the stability specifications and no noteworthy changes or trends over the storage period were observed. The container used for stability studies is composed of the same material as that used for the commercial product, but only smaller in size which is acceptable.

The requested information on PPQ batch stability data has been provided by the Applicant. The to-date submitted data are well within pre-specified acceptance criteria.

The data presented to date are deemed acceptable.

A forced degradation study showed consistent increase in acidic and basic variants as well as HMWs and LMWs species and stability over ten freeze thaw cycles in all investigated batches. During a photostability study the Applicant showed that ENZ215 active substance exposure to UV light and combined visible followed by UV light showed sensitivity to light, as differences in purity were detected.

2.3.3. Finished Medicinal Product

2.3.3.1. Description of the product and Pharmaceutical Development

Description of the product

The finished product (ENZ215 finished product) contains denosumab as an active substance and glacial acetic acid, sodium hydroxide, sorbitol, polysorbate 20 and WFI as excipients (Table 1).

The excipients in the finished product 60 mg-PFS comply with compendial requirements (Ph. Eur./USP). No excipients of human or animal origin, and no novel excipients are used in the manufacture of the finished product.

ENZ215 finished product is a colourless to pale yellow solution, clear solution filled in a 1 mL long Type-1 glass PFS. The PFS (ENZ215 finished product) contains a single dose of 60 mg for subcutaneous injection. The pH of ENZ215 finished product is 5.0 to 5.5. ENZ215 finished product has the same qualitative composition as that of the RMP.

The PFS for administration of Osqay is made from Type I glass with stainless steel 27-gauge 1/2” 3B needle, with a plunger stopper (bromobutyl rubber) and needle safety device.

Table 1. Composition of the finished product

Sr. No.	Ingredients	Pharmacopoeial Reference
1.	Denosumab	In-house
2.	Sorbitol	Ph. Eur. and USP
3.	Polysorbate 20	Ph. Eur. and USP
4.	Sodium hydroxide	Ph. Eur. and USP
5.	Glacial acetic acid	Ph. Eur. and USP
6.	Water for Injection (WFI)	Ph. Eur. and USP

Note: Sodium hydroxide is used for pH adjustment

Formulation development

The formulation development of ENZ215 finished product was performed considering the choice of excipients and ranging study. The ranging study is performed to assess the impact of different ranges of critical input parameters. All the process parameters were evaluated for their risk on the quality and stability of ENZ215

finished product using FMEA approach. For the ranging study, three factors (pH, protein concentration, polysorbate 20 concentration) and two levels (high, low) along with centre point (for each ranging parameter) were considered.

A freeze-thaw study (using ENZ215 active substance) and thawing time (using ENZ215 active substance placebo solution) were carried out to determine freeze-thaw cycle and thawing time of active substance. Based on the observation and results of freeze-thaw study there was no significant change observed in the quality attributes and biological activity of ENZ215 active substance.

In conclusion, the chosen formulation is suitable.

Process characterisation

Process characterisation of ENZ215 finished product was performed. Buffer and process intermediate hold time studies of ENZ215 finished product formulation buffer and formulation bulk solution were executed to support respective hold time at manufacturing scale.

The operating parameters (process parameters) are classified.

The minimum and maximum value of each operating parameter, at which the product quality attributes are proven to be within the acceptable range. The steps involved in the ENZ215 finished product manufacturing and operating parameters of the respective steps are sufficiently discussed.

A hold time study was performed to establish hold time of formulation buffer and FBS at each stage of manufacturing process under specified conditions without impact on product quality attributes.

2.3.3.2. Manufacture of the product and process controls

Manufacture

All sites involved in manufacturing and control of the finished product operate in accordance with EU GMP.

The flow chart of each manufacturing process includes the CPPs and IPCs and testing parameters. The process parameters were classified as CPPs and NCPPs after the process characterisation studies. PARs, ORs and set points were defined.

ENZ215 finished product is manufactured according to a standard process The filled PFS are appropriately labelled and 100 % inspection of labelled PFS is performed. After that, plunger rod is screwed up into the plunger stopper which is further assembled with safety device. The PFS assembled with safety device.

There is no reprocessing proposed/plan in the manufacturing of ENZ215 finished product (PFS).

A batch numbering system to establish the traceability and identification of materials manufactured in the facility is established and presented.

Process controls

The control of critical steps and intermediates section describes the process controls in place.

Process validation

PPQ was performed following a traditional approach. For the ENZ215 finished product process validation, three consecutive batches were manufactured at the Enzene manufacturing facility.

All PPQ batches met the prospective acceptance criteria and in-process controls, and pre-defined specifications.

The protocol and report of cleaning validation study are provided and is acceptable.

Hold time was established for formulation buffer and formulated bulk solution at different stages of manufacturing process.

Overall, based on the available microbial and analytical data the hold time study performed is acceptable for the finished product.

The aseptic process simulation (media fill) performed in the fill finish facility was found satisfactory, demonstrating that the aseptic process and associated system are capable of producing a sterile finished product.

A shipping validation was performed. It could be shown that there were no significant changes in the product's quality attributes were within acceptable limits.

In summary, PPQ data demonstrated that the manufacturing process, when it is operated within the established parameters, produce an effective and reproducible medicinal product, which meets the predetermined specifications and quality attributes.

2.3.3.3. Product specification, analytical procedures, batch analysis

Specifications

Specifications for the finished product include control of identity, purity and impurities, potency and other general tests.

The specification limits to confirm the quality of ENZ215 finished product are set based on process and product knowledge and the data of release analysis and stability results trends of developmental as well as at scale batches.

Specifications were defined considering ICH Q6B guidance. The specifications have been set on release data of nine finished product batches in PFS, encompassing the development history and including, clinical, ACS batches and PPQ batches.

The list of quality attributes and their justifications proposed for ENZ215 finished product release testing is acceptable. Most of the assays of these quality attributes are compendial.

The specifications are adequately described, justified and are acceptable.

Analytical methods

Physical appearance, pH, osmolality, extractable volume, visible and subvisible particulate matter, sterility, deliverable volume and visual inspection are compendial methods. These methods are verified.

Protein concentration by OD280nm, identity and estimation of potency by in vitro bioassay, identity and purity by SE-HPLC, purity by CE-HPLC, BET and polylobate 20 content are in-house methods. These methods are sufficiently validated.

Batch Analyses

The results for batch analyses of batches manufactured at the commercial site are presented. All results of all tested finished product batches met the acceptance criteria listed in the specification at the time of the release. The provided batch data confirm ENZ215 finished product manufacturing process consistency.

Reference standard

The IRS was prepared from the developmental ENZ215 active substance batch and hence it is referred as developmental internal reference standard (dIRS). The dIRS was used as a reference standard for the batch analysis of the finished product batches. Subsequently, pIRS was established Once pIRS is established the pIRS is used for the batch analysis of ENZ215 finished product batches. The detailed description and development of the in-house reference standard is discussed in active substance part of the dossier.

Characterisation of impurities

The finished product is evaluated for bacterial endotoxin and sterility as a part of release testing of finished product batches. The process- and product-related impurities of ENZ215 finished product are evaluated at the active substance level. No elements were intentionally added in the ENZ215 finished product manufacturing process.

A nitrosamine impurities risk assessment was performed and all the nitrosamine analytes (nitrosamine impurities) in ENZ215 active substance and ENZ215 finished product batches were below detection limits.

The starting material, raw material, equipment, consumable and container closure used during the manufacturing of ENZ215 finished product are assessed to identify the risk for the presence of elemental impurities in accordance with ICH Q3D. Based on the risk assessment, there are multiple potential sources of elemental impurities in finished products. Based on the risk assessment an in process leachable study was carried out to cover the product contact parts which have high risk. The leachable study of the finished product batches filled in primary container closure system was done. Results of this study showed that all leachable which were detected with exposure level are well below PDEs.

Container closure system

The Applicant provided a Notified Body Opinion (NBOp) for the PFS confirming full compliance with the relevant general safety and performance requirements (GSPRs).

The Applicant confirmed that the primary container closure system is in compliance with the relevant Ph. Eur. Chapters.

The Applicant performed a risk assessment on container closure system for extractables and leachables and further performed an extractable study on primary container closure system as per USP <1663> and a leachable study as per USP <1664>. Overall, submitted data of the extractable and leachable study of the primary container closure system showed that all extractables and leachable which were detected with exposure levels are well below their PDEs.

2.3.3.4. Stability of the product

The Applicant proposes a 36-month shelf life for ENZ215 finished product when stored at 5°C ± 3°C, protected from light.

Overall, a shelf life of 36 months for the finished product when stored at 2°C – 8°C protected from light is acceptable. However, the Applicant is recommended to submit the long-term stability data for batches. The Applicant commits to inform the authorities immediately if an OOS event occurs for the remaining duration of the stability study.

After approval, the Applicant committed to initiate real-time / long-term stability study for one finished product batch annually, unless there is no production in that year.

A stress study was conducted at 40°C ± 2°C / 75 ± 5 % RH for one development batch for 28 days. All the quality parameters result for the batch are within the specification for 28 days.

Additional stability studies were performed for different conditions such as temperature excursion, thermal cycling and photostability.

Once removed from the refrigerator, Osqay may be stored at room temperature (up to 25°C) for up to 30 days in the original carton. It must be used within this 30-day period.

2.3.3.5. Biosimilarity

A) Analytical comparability exercise

ENZ215 is a proposed biosimilar to Prolia. Analytical similarity of Osqay was assessed in a comprehensive analytical similarity exercise using EU-approved Prolia as RMP.

The similarity assessment is well presented in the dossier. Tables and figures summarising the individual results and data distribution for each parameter, chromatographs, spectra, dose-response curves etc. have been included.

Quality attributes of denosumab were ranked (none, low, moderate, high, and very high risk) based on a risk assessment that considered potential impacts on clinical performance (i.e. bioactivity, pharmacokinetics (PK), safety and immunogenicity) and the degree of uncertainty. A summary of the criticality assignment with brief justifications for the risk ranking has been provided. The outcome of the risk assignment is reasonable. Brief method summaries on used analytical methods for quality attribute comparative assessment have been provided.

- **Approach for data analysis**

Quality attributes included in the comparative assessment cover the relevant attributes of the products. The applicant used a risk ranking score matrix for criticality assessment of quality attributes. Based on criticality score quality attributes were assigned. Criticality assessment and risk ranking are considered sufficiently described and outcome of the risk assessment is considered in general acceptable.

As regards statistical approach, for each attribute a pre-planned multiplier x was defined to be used in constructing the mean + x SD limits of acceptable values of the test product data set based on results from the reference product. Similarity criteria were set for quality range (QR), the data are considered highly similar.

Overall, the sourcing of batches is considered meaningful and covers a broad range of batch ages and development stages.

The number of lots is expected to reflect sufficiently variability and hence, deemed acceptable for evaluation of similarity.

The selected comprehensive set of orthogonal state-of-the–art analytical methods, which covers primary and higher order structure, size, glycoform and charge variants, post-translational modifications, protein concentration, as well as multiple biological functions mediated by the RANKL binding/inhibition of its actions. They are adequate to address the relevant quality attributes of denosumab.

- **Obligatory CQAs**

Protein content

The data obtained by OD280nm is evaluated for similarity between ENZ215 finished product and Prolia with the result that ENZ215 finished product protein content by OD280nm is similar to EU approved Prolia.

Reporter Gene Assay using HEK Blue RANKL cells

To assure comparability in the primary mechanism potency of ENZ215 and EU approved Prolia samples was assessed using reporter gene assay with HEK BlueTM RANKL cells. The mean difference between between EU approved Prolia and ENZ215 samples was found to be within pre-set acceptance criteria. Similarity regarding binding of RANKL is confirmed.

- **Charge variants**

Charged variants of EU approved Prolia and ENZ215 were investigated by CE-HPLC and cIEF. For all ENZ215 batches, % of main peak by CE-HPLC was within acceptance limits. Acidic variants were not detectable with the release method, but were detectable with the characterisation method, which achieved greater separation between acidic and basic variants. Here, acidic variants were comparable between Prolia and ENZ215. Basic variants were comparable between Prolia and ENZ215 by CE-HPLC.

- **Size variants and purity analysis**

Size variants were evaluated by size exclusion chromatography (SEC), non-reducing/reducing CE-SDS and RP-UPLC.

Aggregates were not detected in both ENZ215 finished product & Prolia using SE-HPLC release method. However, CE-SDS (non-reduced) analysis showed in majority of ENZ215 finished product batches slightly higher % of HMWs and LMWs content which was concluded to be similar between ENZ215 finished product & EU-Prolia The applied comparability study along with so far long-term stability data demonstrated that the observed differences in size variants are minor, consistent over time, and do not impact product quality, safety, or efficacy. Based on the available data the commercial finished product can be considered comparable to EU-Prolia with respect to size-related variants.

- **Post-translational modifications**

Isomerisation of Aspartic Acid in HC and LC CDRs, Oxidation at Methionine in HC and LC CDR (HC: M106), Oxidation at Methionine in HC and LC non CDRs (HC: M34), Oxidation at Methionine in HC and LC non CDRs (HC: M83), Oxidation at Methionine in HC and LC non CDRs (HC: M253) and Oxidation at Methionine in HC and LC non CDRs (HC: M398) were comparable between EU approved Prolia and ENZ215 batches by Peptide mapping analysis by UPLC MS/MS. In contrast, Oxidation at Methionine in HC and LC non CDRs (HC: M429) by Peptide mapping analysis by UPLC MS/MS was not considered similar as 2 batches of ENZ215 were not measured within the acceptance limit. For these batches higher oxidation was discussed by the Applicant. As this oxidation is not within the CDR region and the two batches were only marginally above the acceptance threshold (0.1 %), the differences were considered negligible, although as per statistics this attribute is not comparable. The argumentation of the Applicant could be followed without further questions.

The following was analysed by peptide mapping analysis by UPLC-MS/MS and similarity was demonstrated between EU approved Prolia and Osqay batches

Glycation of LC and HC was compared by reduced mass analysis with all Osqay batches lying within acceptance criteria. Trisulfide analysis by limited proteolysis was also indicative of similarity.

Disulfide isoforms were analysed by RP-HPLC. Average Disulfide Isoform distribution is comparable.

Lysine variants by Peptide mapping analysis UPLC-MS/MS, Dimers & Monomer Purity % by SE-HPLC, Acetate content by RP-HPLC, Sorbitol content analysis by ion exclusion, Polysorbate 20 analysis by HILIC were found to be similar between all investigated Osqay batches and EU Prolia.

Overall post translational modifications could be deemed comparable.

- **Functional assays**

An extensive comparability of functional assays including biological activity (inhibition of osteoclastogenesis), binding to sRANKL and mRANKL, binding to FcRn, FcγR receptors, c1q as well as ADCC and CDC have been explored and documented. No differences were observed between ENZ215 and EU-Prolia. In addition, binding to FcγRIIa and to FcγRIIb/c has been assessed, including both polymorphic variants of FcγRIIa,. No differences are found in FcγRIIa and FcγRIIb/c binding, thus no evaluation of ADCP was performed. No ADCC activity was detected. Weak CDC-based activity was detected for both ENZ215 and EU-Prolia and was comparable between tested items. Since not all batches were included in comparative in vitro studies to evaluate binding affinity of ENZ215 and EU-Prolia to RANKL by SPR and to evaluate complement-dependent cytotoxicity (CDC) activity. Together with method suitability information and additional supportive characterisation data on CDC activity, it is considered that batches evaluated by SPR and CDC assay support the biosimilarity conclusion with regard to biological activity.

- **Primary, secondary and tertiary structure**

Peptide map analysis was performed to compare amino acid sequence of ENZ215 finished product and Prolia RMP. Results showed % sequence coverage of ENZ215 batches, PPQ batches in comparison to EU Prolia. It could be demonstrated that a complete match of the theoretical amino acid sequence of denosumab is present.

The intact mass (glycosylated and deglycosylated) of ENZ215 and EU-Prolia was analysed. Average glycosylated intact masses of EU-Prolia and ENZ215 finished product are 147356 Da and 147355 Da respectively, average deglycosylated intact masses of EU-Prolia and ENZ215 finished product are 144467 Da and 144466, respectively, reduced mass analysis of glycosylated and deglycosylated Prolia and ENZ215 finished product as well as average masses of Fc/2 and F(ab')2 showed comparable results between ENZ215 finished product and Prolia.

All expected 18 disulfide linkages(s) were idendified and confirmed by LC-MS/MS-based analysis. Position and mass of disulfide linkages of ENZ215 finished product is identical with EU approved Prolia.

Comparable secondary and tertiary structures of ENZ215 and EU-Prolia were confirmed by FAR-UV CD spectroscopy, fluorescence spectroscopy and near-UV CD spectroscopy.

Free thiols were determined justified minor difference in the level of free thiol between Osqay and EU Prolia to be due to method and batch to batch variability and are not clinically meaningful which is acceptable.

- **Glycan analysis**

With reference to glycans, glycan analysis by normal phase HILIC revealed that ENZ215 and EU-Prolia are not similar in terms of afucosylation and galactosylation.

Osqay shows lower afucoslyation than EU Prolia. Osqay shows slightly lower galactosylation than EU Prolia.

The Applicant discussed these differences in glycosylation with the determination that these differences in glycosylation do not have an impact as denosumab does not bring out effector functions. However, the Applicant further discussed on impacts on glycosylation and performed ADCC and CDC assays. This extensive evaluation

and discussion on differences is considered meaningful and is endorsed. Thus, for the mode of action of the denosumab molecule, these differences are negligible.

In terms of agalactosylation, High Mannose, Sialylation and total Fucosylation the results were found to be similar for Osqay and EU-Prolia.

- **General characteristics**

Sub-visible particulate matter using micro-flow imaging (MFI), pH and extractable volume using a gravimetric method were found to be similar by visual comparison.

It could be convincingly shown with a state-of-the-art analytical comparison that Osqay is highly similar to EU-Prolia in terms of functional and structural attributes, indicative of biosimilarity.

- **Comparative forced degradation study**

As part of the analytical comparability, comparative degradation of three EU approved Prolia and three Osqay finished product batches was carried out. Sourcing of batches is suitable. These batches were exposed to high and low pH, oxidating and reducing agents, mechanical degradation using a shaker, freeze thawingcycles, thermal degradation above the stress stability temperature (60°C) and photo-degradation under visible, UV- and both wavelengths combined. Measurements were performed with standard methodology and compared side-by-side.

Based on the presented results of the forced stress studies, it can be concluded that ENZ215 and EU-Prolia are comparable in terms of degradation. Overall, the data substantiate the similarity.

B) Conclusion on biosimilarity

A comprehensive assessment of biosimilarity between ENZ215 PFS and EU-Prolia has been presented. The analytical similarity assessments included a similarity analysis (Table 2) of primary and higher order structure, purity/impurity, content, glycan profiles and post-translational modifications, as well as biological assays. The observed differences have been assessed and concluded not to be significant in terms of comparability assessment. Osqay is considered biosimilar to Prolia from a quality point of view.

Table 2. Summary of the Quality Attributes and Analytical Similarity Acceptance Criteria

Sr. No.	Quality Attributes	Analytical Method	Conclusion
Obligatory CQAs			
1	Protein Concentration	OD 280nm	Similar
2	Biological activity (cell-based assay)	Reporter gene assay using HEK Blue RANKL cells	Similar
Charge Variants			
3	%Main peak	CE-HPLC (Cation exchange	Similar
4	%Total acidic variants		Similar

Sr. No.	Quality Attributes	Analytical Method	Conclusion
5	%Total basic variants	chromatography): Release	Similar
6	%Main peak	CE-HPLC (Cation exchange chromatography): Characterization	Similar
7	%Total acidic variants		Similar
8	%Total basic variants		Similar
9	%Main peak	cIEF	Not similar
10	%Total acidic variants		Not similar
11	%Total basic variants		Similar
12	pI of Main peak		Similar
Size Variants and Purity analysis			
13	Aggregates (HMWs)	Size Exclusion Chromatography	Similar
14	% Monomer	CE-SDS (Non-reduced)	Similar
15	% HMWs		Similar
16	% LMWs		Similar
17	% NGHC (Aglycosylated Product)	CE-SDS (Reduced)	Similar
18	% Other LMWs		Similar
19	% LC + HC		Similar
20	D/P clips N-terminal	RP-UPLC	Similar
21	D/P clips C-terminal		Similar
Post Translational Modifications			
22	Isomerization of Aspartic Acid in HC and LC CDRs	Peptide mapping analysis by UPLC MS/MS	Similar
23	Oxidation at Methionine in HC and LC CDR (HC: M106)		Similar
24	Oxidation at Methionine in HC and LC non CDRs (HC: M34)		Similar

Sr. No.	Quality Attributes	Analytical Method	Conclusion
25	Oxidation at Methionine in HC and LC non CDRs (HC: M83)		Similar
26	Oxidation at Methionine in HC and LC non CDRs (HC: M253)		Similar
27	Oxidation at Methionine in HC and LC non CDRs (HC: M398)		Similar
28	Oxidation at Methionine in HC and LC non CDRs (HC: M429)		Not similar
29	Succinimide at Asparagine in HC and LC non CDRs (HC: N316)	Peptide mapping analysis by UPLC-MS/MS	Similar
30	Succinimide at Asparagine in HC and LC non CDRs (HC: N385)		Similar
31	Deamidation at Asparagine in HC and LC non CDRs (HC: N316)		Similar
32	Deamidation at Asparagine in HC and LC non CDRs (HC: N362)		Similar
33	Deamidation at Asparagine in HC and LC non CDRs (HC: N385)		Similar
34	Deamidation at Asparagine in HC and LC non CDRs (HC: N390/N391)		Similar
35	Deamidation at Asparagine in HC and LC non CDRs (HC: N422/N435)		Similar
36	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D61)		Similar
37	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D71)		Similar
38	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D83)		Similar
39	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D123)		Similar
40	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D152/D168)		Similar

Sr. No.	Quality Attributes	Analytical Method	Conclusion
41	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D171)		Similar
42	Isomerization of Aspartic Acid in HC and LC non CDRs (LC: D186)		Similar
43	Isomerization of Aspartic Acid in HC and LC non CDRs (HC: D90)		Similar
44	Isomerization of Aspartic Acid in HC and LC non CDRs (HC: D217)		Similar
45	Isomerization of Aspartic Acid in HC and LC non CDRs (HC: D400/402)		Similar
46	Isomerization of Aspartic Acid in HC and LC non CDRs (HC: D414)		Similar
47	N-terminal glutamic acid to pyroglutamic acid in LC		Similar
48	N-terminal glutamic acid to pyroglutamic acid in HC		Similar
49	Glycation in LC	Reduced mass analysis (deglycosylated)	Similar
	Glycation in HC		Similar
50	Trisulfide (One sulfur addition within disulfide bridge)	Limited Proteolysis	Similar
51	Disulfide Isoforms	RP-HPLC	Similar
52	Lysine Variants	Peptide mapping analysis by UPLC-MS/MS	Similar
53	Dimers & Monomer Purity %	SE-HPLC	Similar
54	Acetate content analysis	RP-HPLC	Similar
55	Sorbitol content analysis	Ion exclusion	Similar
56	Polysorbate 20 analysis	HILIC	Similar
Functional Assays			

Sr. No.	Quality Attributes	Analytical Method	Conclusion
57	Biological activity (cell-based assay)	Inhibition of osteoclastogenesis cell-based assay	Similar
58	Fab based Binding assays	Binding to sRANKL by ELISA	Similar
59		Binding to mRANKL by ELISA	Similar
61	Binding of Fc to Fc receptors	Binding to FcRn by BLI	Similar
62		Binding to FcγRIIa by BLI	Similar
63		Binding to FcγRI by BLI	Similar
64		Binding to FcγRIIIa (F158) by BLI	Similar
65		Binding to FcγRIIIa (V158) by BLI	Similar
66		Binding to c1q by ELISA	Similar
67	Fc based Cell based assays	ADCC	Similar
68		CDC	Similar
Structural Analysis			
69	Primary Structure Analysis	Intact mass analysis (Glycosylated)	Similar
		Intact mass analysis (Deglycosylated)	Similar
70		Reduced mass analysis (Glycosylated)	Similar

Sr. No.	Quality Attributes	Analytical Method	Conclusion
		Reduced mass analysis (Deglycosylated)	Similar
71		Limited Proteolysis	Similar
72		Peptide mapping analysis by UPLC-MS/MS	Similar
73	Disulfide bridge formation	Peptide mapping analysis by UPLC-MS/MS under non-reducing condition	Similar
74	Higher order structure	Far-UV CD spectroscopy	Similar
75		Fluorescence spectroscopy	Similar
76		Near-UV CD spectroscopy	Similar
77		DSC analysis	Similar
78		AUC	Similar
79		SEC MALS	Similar
82	Free Thiol Estimation	Elman's assay	Similar
Glycan Analysis			
83	Afucosylation	Glycan Analysis by HILIC	Not similar
84	Galactosylation		Not similar
85	Agalactosylation		Similar
86	High Mannose		Similar
87	Sialylation		Similar
88	Total Fucosylation		Similar

Sr. No.	Quality Attributes	Analytical Method	Conclusion
General Characteristics			
89	Sub-visible particulate matter using MFI	MFI	Similar
90	pH	pH meter	Similar
91	Extractable Volume	Gravimetric method	Similar

2.3.3.6. Adventitious agents

No raw materials and excipients of animal origin are used in the manufacturing of ENZ215 active substance and finished product. Adequate TSE/BSE declarations for the raw materials including contact parts of the approved vendors were provided.

MCB, WCB and EPCB were tested for the absence of bacterial and fungal contamination (sterility and mycoplasma) in accordance with the ICH Q5A(R2) and Q5D guidelines.

Adventitious agent contamination was evaluated in the unprocessed bulk during upstream process development. The unprocessed bulk for the 3 PPQ batches were presented in the dossier.

Routine testing for bioburden, mycoplasma, *in vitro* adventitious virus, and MVM qPCR, with acceptance criteria, was included for the release of unprocessed bulk harvest lots in accordance with Ph. Eur. and ICH Q5A(R2).

Robust and effective overall virus clearance by three orthogonal manufacturing process steps was demonstrated. Two dedicated virus clearance steps, i.e. low pH treatment and virus filtration, as well as the AEX chromatography step effectively reduced enveloped and non-enveloped viruses.).

The estimated number of retrovirus-like particles (RVLP) per dose was calculated in accordance with the ICH Q5A(R2) guideline, which is considered acceptable for monoclonal antibodies produced in CHO cells.

In summary, the risk of potential contaminations and transmission of bacterial, viral, or TSE agents is adequately controlled and acceptably low.

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

Active substance

ENZ215 has been developed as a similar biological medicinal product (biosimilar) to Prolia, having denosumab as the active substance. The manufacturing process is a standard process for recombinant protein production and is adequately described.

Information on used raw materials used in upstream cell culture and downstream processing including certificates and filter details are sufficiently provided.

The overall control strategy of the relevant CQAs including risk assessment tools is described. The methodology as well as the proposed classification of quality attributes in critical and non-critical attributes can be agreed. The IPCs and their acceptance criteria/action limits are considered adequate and sufficiently described.

Process characterisation and process verification (PPQ) data from three consecutive PPQ batches at 500 L manufacturing scale support the conclusion that the active substance manufacturing process reliably generates active substance meeting its predetermined specifications and quality attributes.

Overall, process comparability could be shown and hold times are listed and sufficiently justified for the respective manufacturing steps.

A comprehensive characterisation of structural and functional features of ENZ5121 has been performed based on broad panel of standard and state-of-the-art methods. In addition, a discussion of the potential impurities in ENZ5121 active substance has been provided.

The active substance specifications are acceptable. The Applicant is recommended to implement via a variation cIEF or strong CEX for control of charge variants in the active substance and finished product (REC1).

Overall, the analytical methods are appropriately validated. The Applicant is recommended to consider the feasibility of transitioning to Ph. Eur. 2.6.32 Test for bacterial endotoxins using recombinant factor C in the future (REC2).

Reference standards are described and characterised. The container closure system is suitable and as requested some elements of the technical drawing were updated.

Based on the submitted stability data, the proposed shelf-life of 24 months when stored at -20°± 5°C, protected from light is acceptable.

Finished product

ENZ215 finished product is presented as a single-dose PFS containing 60 mg/1.0 mL of denosumab for subcutaneous injection. Excipients used are of compendial quality. Quality and quantitative composition for ENZ215 finished product is the same as the active substance but is diluted with formulation buffer to achieve the concentration required to manufacture ENZ215 finished product.

Sufficient documentation on the finished product development (formulation, manufacturing process development from Process-1 and commercial Process-2), manufacturing, characterisation, control and stability has been provided.

Overall, release and stability results of the ENZ215 finished product batches manufactured at development R&D site and commercial site Enzene Bioscience Limited, India with Process-1 (clinical batch) and Process-2 (PPQ batches) showed comparable data.

PPQ data demonstrated that the manufacturing process, when it is operated within the established parameters, produce an effective and reproducible medicinal product, which meets the predetermined specifications and quality attributes.

The Applicant confirmed that the primary container closure system is in compliance with the relevant Ph. Eur. Chapters. The Applicant provided a NBOp for the PFS confirming full compliance with the relevant GSPRs.

The Applicant performed a risk assessment on container closure system for extractable and leachable and further performed an extractable study on primary container closure system. Overall, submitted data of the extractable and leachable study of the primary container closure system showed, at the moment, that all extractables and leachable which were detected with exposure level are well below their PDEs. The Applicant

is recommended to submit a summary of the data after 36-month timepoint of leachable study at real time condition for finished product batches (REC3).

The proposed finished product specifications are acceptable.

An elemental impurities risk assessment and nitrosamine impurities risk assessment were performed and are satisfactory.

The Applicant proposed a shelf life for ENZ215 finished product of xx 36 months when stored at 5°C ± 3°C, protected from light. This is acceptable. The Applicant is recommended to submit the long-term stability data for batches for 36 xx-month timepoint (REC4).

Additional stability studies were performed for different conditions such as temperature excursion, thermal cycling and photostability. The stability data for these additional stability studies are acceptable.

Biosimilarity

A very comprehensive and sound biosimilarity assessment has been conducted. A broad panel of orthogonal state-of-the-art methods has been applied for biosimilarity evaluation to address general properties, primary structure, secondary, tertiary and higher order structure, post-translational modifications, product purity, and biological activity. Degradation profiles have been analysed in comparative stability studies. All individual test results of the analytical similarity exercise are provided and based on the provided information; it is concluded that the analytical methods are suitable for the intended purpose.

The provided results support the biosimilarity claim. For most of the quality attributes similarity was demonstrated, observed differences in certain quality attributes are minor and are sufficiently justified to have no impact on the clinical performance of the product.

Adventitious Agents

The risk of potential contaminations and transmission of bacterial, viral, or TSE agents is adequately controlled and minimised by the complementary measures implemented at various stages of the manufacturing process.

Overall, the quality of this product is considered acceptable when used in accordance with the conditions defined in the SmPC. Physico-chemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

In conclusion, based on the review of the quality data provided, the marketing authorisation application for Osqay is considered approvable from the quality point of view.

2.3.5. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The Applicant is recommended to implement via a variation cIEF or strong CEX for control of charge variants in the active substance and finished product.

2. The Applicant is recommended to consider the development of a method of recombinant Factor C (rFC)-based bacterial endotoxin. Upon successful completion of method validation, implementation of the method would require a post-approval variation.

3. The Applicant is recommended to submit a summary of the data after 36-month timepoint of leachable

study at real time condition for finished product batches FC21512201, FC21512301, and FC21512302.

4. The Applicant is recommended to submit the long-term stability data for batches FC21512301 and FC21512302 for 36-month timepoint.

2.4. Non-clinical aspects

2.4.1. Introduction

Osqay is under development as a biosimilar to Prolia, which contains 60 mg denosumab monoclonal antibody as active ingredient presented as a solution in a pre-filled syringe for injection under the skin. The active substance (denosumab) is a human monoclonal antibody of the IgG2 subtype that inhibits the interaction of receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) with RANK on the surface of osteoclasts. This inhibition prevents the development (genesis, maturation, activation and survival) of osteoclasts, the cells responsible for bone resorption that play a critical role in bone modelling and remodelling during growth. Pathological disturbance of this balance towards excessive bone resorption can be counteracted by means of RANKL-inhibition with denosumab.

Aspects of non-clinical development fall within the regulatory scope of EMA/CHMP/BMWP/403543/2010 (Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues), according to which nonclinical in vivo studies are deemed dispensable if no relevant factors (e.g., differences to the RMP in quality attributes or formulation) suggest otherwise. No such factors were identified for Osqay.

Nonetheless, the Applicant has filed three non-clinical studies under Module 4 (Nonclinical study reports) of the eCTD. One pharmacokinetics study is redundant to section 3.2.R in Module 3 (Quality) and shall not be further discussed in this section. Two GLP-compliant *in vivo* toxicology studies in rats and rabbits were also presented. The Applicant points out that the studies were performed to meet regulatory requirements imposed by the Indian national authority. The respective study reports are filed alongside the European MAA for information purposes only and would only affect the outcome of this procedure in the unexpected event of obvious toxicological risks associated with the product. No such events were identified in both repeated dose toxicity studies. Hence, the provided studies should not affect the outcome of the present European MAA in any way.

2.4.2. Ecotoxicity/environmental risk assessment

The Applicant states that "in line with the "Guideline on the Environmental Risk Assessment for medicinal products for human use" (EMEA/CHMP/SWP/4447/00 Rev. 1), amino acids, peptides and proteins are unlikely to result in significant risk to the environment. There is no environmental risk assessment required. Denosumab has similar physicochemical properties to the antibodies/proteins found in human body and post metabolism it gets released into the environment in the form of amino acids. The amino acids do not pose a risk to the environment. As per the guideline there is no need for an ERA and hence there is no further risk assessment carried out."

2.4.3. Discussion on non-clinical aspects

In line with the Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues (EMA/CHMP/BMWP/403543/2010), non-clinical in vivo studies are deemed dispensable if no relevant factors (e.g., differences to the RMP in quality attributes or formulation) suggest otherwise. No such factors were identified for Osqay. Nonetheless, the applicant submitted three non-clinical studies, which are considered supportive for the purpose of this application.

Environmental risk assessment

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, denosumab is not expected to pose a risk to the environment.

In conclusion, the marketing authorization application for Osqay is considered approvable based on non-clinical aspects.

2.4.4. Conclusion on the non-clinical aspects

The CHMP considers that the MAA for Osqay is approvable from a non-clinical perspective.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

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

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

- **Tabular overview of clinical studies**

Table 1. Tabular overview of clinical studies

Study Type, Identifier and Status	Location of Study Report	Objective(s) of the study	Study Design and Type of control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy subjects or Diagnosis of patients	Duration of Treatment
PK/PD Project No. ALK22/ENZ215-DEN1 Completed	CSR Module 5.3.1.2	**Primary objective:** • To demonstrate bioequivalence between ENZ215 and EU- and US-sourced Prolia using PK parameters. **Secondary objective:** • To compare the serum PK profile, serum CTX-1 profile, immunogenicity profile, safety and tolerability profile of ENZ215 and EU- and US-sourced Prolia	**Study Design:** Phase I, randomised, double-blind, three-arm, parallel-group, and single-dose study. **Type of Control:** Active control	**Test Product-T** ENZ215 (denosumab) injection 60 mg, single dose, SC **Reference Product 1** EU Prolia (denosumab) injection 60 mg, single dose, SC **Reference Product 2** US Prolia (denosumab) injection 60 mg, single dose, SC	Planned-207 (69 subjects per arm) Dosed-206 Analysed-206 Completed-200 Discontinued/ Withdrawn-7	Healthy subjects	Single dose
Efficacy/Safety/PK/ PD/ADA Project No. ALK22/ENZ215-DEN2 Completed	Clinical Study Report 5.3.5.1	**Primary Objective:** • To evaluate the efficacy of ENZ215 when compared to Prolia in participants with postmenopausal osteoporosis, in terms of change in BMD at the lumbar spine from baseline to Month 12 and • To compare the AUEC of sCTX levels from baseline to Month 6 **Secondary Objective:** • To compare the change in sP1NP levels, the change in BMD at lumbar spine from baseline to Month 6. • To compare the change in BMD at total hip and femoral neck from baseline to Month 6 and Month 12 • To compare the immunogenicity potential, the safety and tolerability of ENZ215 and Prolia	**Study Design:** Phase 3, Randomised, double-blind, active-controlled, parallel-arm, multicenter study of ENZ215 with Prolia in postmenopausal women with osteoporosis **Type of Control:** Active control	**Test Product-T** denosumab 60 mg/mL Solution for injection in single-use prefilled syringe **Reference Product-R** EU Prolia® 60 mg/mL Solution for injection in single-use prefilled syringe **Route of administration**: Subcutaneous	**Treatment Period:** Planned- 504 Dosed- 504 Completed (clinical phase)- 469 Drop-out: 35 Analysed: ITT set: 504 PK- 116 PD- 471 **Open-label extension Period:** Planned- 120 Dosed- 120 Completed (clinical phase)- 120 Drop-out: 0	Postmenopausal women with osteoporosis between the ages of 55 to 90 years	Single dose repeated at 6 months for all patients and again at 12 months for patients entering the Transition-Extension Period

2.5.2. Clinical pharmacology

The clinical pharmacology of ENZ215 and the reference product has been investigated in two studies:

- Clinical Phase I study (ALK22/ENZ215-DEN1): A randomized, double-blind, three-arm, parallel-group, single-dose study to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and

immunogenicity of Denosumab (ENZ215, EU-sourced Prolia, and US-sourced Prolia) in healthy adult male volunteers

- Clinical Phase III study (ALK22/ENZ215-DEN2): A Phase 3, Randomised, Double-blind, Parallel-group, Active-controlled Study to Compare the Efficacy, Safety, Pharmacodynamics, Pharmacokinetics and Immunogenicity of Enzene Denosumab (ENZ215) and Prolia in Postmenopausal Women with Osteoporosis.

Apart from the above-mentioned studies, no other clinical pharmacology studies (i.e., drug interaction studies, or studies in special populations such as hepatic or renal impairment) were performed as these are not required for biosimilars.

2.5.2.1. Pharmacokinetics

Bioanalytical methods

Determination of Denosumab in Human Serum by Using an ELISA Assay

The Applicant has adopted a direct enzyme- linked immunosorbent assay (ELISA) to quantitate ENZ215 and EU/US- Prolia (denosumab). The presented assay for determination of denosumab in human serum of healthy volunteers and patients with osteoporosis was well described and established. It could be considered valid for its intended use.

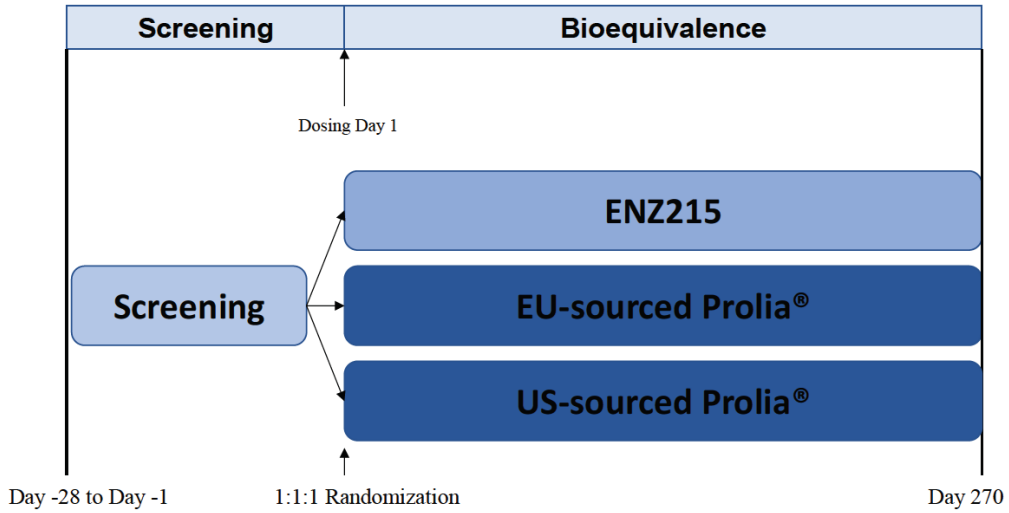
2.5.2.1.1. ALK22/ENZ215-DEN1 (main PK study)

Clinical Phase I study (ALK22/ENZ215-DEN1): A randomized, double-blind, three-arm, parallel-group, single-dose study to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity of Denosumab (ENZ215, EU-sourced Prolia, and US-sourced Prolia) in healthy adult male volunteers

This study is the pivotal PK study.

Study design

Figure 1. Study Flow Chart



End of Study Assessment was performed on day 270 (week 39) or at the time of early discontinuation/withdrawal of the subject.

Study population

For the assessment of pharmacokinetics, healthy volunteers' populations were chosen as they constitute a homogenous population and are likely to have less variability in PK as target-mediated clearance may be less important than in patients.

A total of 207 healthy male volunteers (69 in each group), aged between 28 to 55 years (both included) and fulfilling the study selection criteria were to be enrolled in this study.

Inclusion criteria:

1. Able to understand and give written, voluntary informed consent for the study
2. Healthy adult male volunteers between 28 to 55 years of age (both inclusive)
3. Body Mass Index (BMI) ≥ 18.50 and ≤ 30.00 kg/m2 at the time of screening
4. Medically healthy with no clinically significant medical history, vital signs, physical examination, and laboratory profiles
5. Normal or clinically acceptable 12-lead electrocardiogram, QT interval corrected for heart rate (QTc interval)* ≤ 450 msec at the time of screening
6. Subjects with negative alcohol test (breath analyzer or any suitable test) at the time of screening and admission (pre-dose)
7. Male subjects with female partners who agree to use effective contraception during study#
8. Male subjects who agree not to donate sperm during study
9. Willing and able to comply with the protocol requirements

10. Willing for multiple sampling and admission at the phase 1 study site day before dosing

*Note: QTc interval will be calculated using the Bazette and Fridericia formula

Effective contraception: Non-vasectomised male volunteers with female partners of child bearing potential should use dual method of contraception i.e. condom with spermicide method of contraception. Female partners should use hormonal or non-hormonal method of contraception. (No restrictions are required for a vasectomised male provided his vasectomy has been performed 4 months or more prior to the first dosing. A male who has been vasectomised less than 4 months prior to the first dosing must follow the same restrictions as a non-vasectomised male).

Exclusion criteria:

1. Known hypersensitivity to Denosumab or to any of the components of the study drug
2. Participating or has received any investigational drug (or is currently using an investigational device) within 30 days before receiving the study drug, or at least 10 times the respective elimination half-life (whichever period is longer; For monoclonal antibody refer exclusion criteria number 18 and 19)
3. A serious infection (associated with housing and/or required intravenous anti-infectives) within 6 months before study drug administration and/or any active infection within 4 weeks of screening requiring oral or systemic antibiotics
4. History of significant drug abuse within 12 months before screening or a use of soft drugs (such as marijuana) within 3 months before the screening visit or hard drugs (such as cocaine, phencyclidine, and crack etc.) within 12 months before screening
5. Smokers who smoke ≥ 10 cigarettes or equivalent per day within 90 days prior to screening
6. Subjects with positive urine screen for drugs of abuse at the time of screening or check-in
7. Subjects with Urine Cotinine > 500ng/ml at the time of screening or check-in
8. Subjects with risk of osteonecrosis of the jaw i.e. poor oral hygiene, periodontal disease, poorly fitting dentures, history of dental disease or have undergone invasive dental procedures e.g. tooth extractions within last 6 months prior to screening.
9. Subjects with a predictable risk of invasive dental surgery during the 9 months after dosing or with planned invasive dental procedure
10. Subjects with known bone disease or recent fracture or abnormalities of calcium metabolism
11. Loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL within 30 days, or more than 499 mL within 56 days before dosing
12. History of immunodeficiency (including those subjects with a positive test for human immunodeficiency virus [HIV] at screening)
13. Have a positive result for hepatitis B antigen test (HBsAg) or hepatitis C antibody test (HCAb), or show evidence of possible infection
14. Major surgical procedure within 28 days of dose of investigational product.
15. Male subjects having pregnant female partner at the time of screening.
16. Subject with a history of recurrent or chronic infections

17. Received live vaccines within 4 weeks or who may require live vaccine(s) during the study duration
18. Prior use of denosumab
19. Have previously been exposed to a monoclonal antibody or fusion protein within 270 days (other than denosumab) prior to randomisation and/or there is confirmed evidence or clinical suspicion of immunogenicity from previous exposure to a monoclonal antibody or fusion protein.
20. Any reason/condition which would preclude subject's participation in the study as per the Investigator's opinion or warnings and contraindications in the prescribing information of Prolia
21. Subjects with suspected signs and symptoms of COVID-19/confirmed novel coronavirus infection (COVID-19).

Discontinuation of Study Intervention

Delay in administering the study intervention for not longer than 28 days is allowed in exceptional cases such as patients diagnosed with any transient disease/SAE/reversible hypocalcaemic condition where IP administration would not be possible or would be contraindicated.

Treatments

A total of 207 healthy male subjects who met the required entry criteria were randomly assigned to one of 3 treatment groups in a 1:1:1 ratio (68 subjects in ENZ215, 70 in US-Prolia and 69 in EU-Prolia) to receive a single subcutaneous (SC) injection of 60 mg on day 1 into the upper thigh.

Table 2. Details of Test Product and Reference Products

IP Details	Test Product (A)	Reference Product (B)	Reference Product (C)
Brand Name	ENZ215	US-sourced Prolia® (denosumab) injection	EU-sourced Prolia® injection (denosumab)
Active Ingredient	Denosumab	Denosumab	Denosumab
Pharmaceutical Dosage Form, Strength, Route and Dose	A single-dose, 60 mg subcutaneous injection administration	A single-dose, 60 mg subcutaneous injection administration	A single-dose, 60 mg subcutaneous injection administration
Manufacturer	Enzene Biosciences Ltd.	Amgen Inc.	Amgen Europe B.V.
Storage Condition	Store in a refrigerator (2°C – 8°C). Do not freeze	Store in a refrigerator (2°C – 8°C). Do not freeze	Store in a refrigerator (2°C – 8°C). Do not freeze

Objectives and endpoints

The primary objective of the study was to demonstrate bioequivalence between ENZ215 and EU- and US-sourced Prolia using PK parameters. The secondary objectives of the study were to compare the serum PK profile, serum CTX-1 profile, the immunogenicity profile, safety and tolerability profile of ENZ215 and EU- and US-sourced Prolia.

Primary endpoints (PK):

- Maximum observed drug concentration (Cmax).
- Area under the drug concentration-time curve from Day 0 to Day 270 (AUC0-t).
- Area under the drug concentration-time curve from time 0 to infinity (AUC0-inf).

Secondary Endpoints (PK):

- Partial area under the drug concentration-time curve from time 0 (pre-dose) to Day 28.
- Time to reach Cmax (tmax).
- Terminal elimination half-life (t1/2).
- Apparent systemic clearance (CL/F).

Secondary Endpoint (PD):

- Area under the effect curve (AUEC) from time 0 to Day 270 for serum CTX-1 percent inhibition.

Secondary Endpoints (Safety and Immunogenicity):

- Number of subjects who developed denosumab neutralizing antibodies and anti-drug antibodies (Day 1, 28, 90, 180, and 270).
- Incidence of adverse events.
- Clinically significant changes in physical examination findings, safety laboratory analyses (serum chemistry, hematology, and urinalysis), vital signs, and 12-lead electrocardiogram (ECG).

PK blood samples were collected at 0 hour (pre-dose), and at 1, 4, 8, and 12 hours (Day 1), Day 2, Day 3, Day 4, Day 5, Day 6, Day 8, Day 10, Day 12, Day 16, Day 21, Day 28 (Week 4), Day 42 (Week 6), Day 63 (Week 9), Day 90 (Week 13), Day 119 (Week 17), Day 147 (Week 21), Day 180 (Week 26), Day 224 (Week 32), and at end of study (Day 270 [Week 39] post-dose).

The CTX-1 PD assessment was performed by estimation of serum CTX-1 concentration at day 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 21, 28, 63, 119, 180 and 270. For CTX-1, blood samples should be collected at the same time (in the morning between 07:30 and 10:00 am) and after a minimum of 10 hours of fasting.

Sample size

It was assumed that the true ratio for AUC0-t, AUC0-inf and Cmax between ENZ215, EU-sourced Prolia and US-sourced Prolia is 0.95 and the between subject coefficient of variation (CV) for AUC0-t, AUC0-inf and Cmax is 33.5%. This gives a sample size of 189 (63 per group) to provide at least 90% power for ensuring that the 90% confidence interval (CI) of the ratio of AUC0-t, AUC0-inf and Cmax between ENZ215 and Prolia

groups will be within the (80% to 125%) limits. Assuming a drop-out rate of approx. 10% (as this study is a long study in healthy volunteers), at least 207 (69 per group) subjects are required.

Randomization

Subjects were planned to receive either ENZ215 or EU- sourced Prolia or US-sourced Prolia once during study in a 1:1:1 ratio, according to a randomization scheme. The randomization scheme did not take into account any stratification factors.

Each subject was assigned a unique identification number upon screening. Subjects who completed the study screening assessments and met all the eligibility criteria were assigned a unique randomization identification number at the time of the dosing, different from the screening number, and were to receive the corresponding study drug.

Randomization could be done either on day 0/ day1, however, IP administration must be done on day 1.

Blinding

This study employed a double-blind design. A computerized randomization scheme was created by a statistician and it was considered blinded (as per the following). The randomization was made available only to the clinic pharmacy staff preparing the drug who were not involved in any other aspect of the study including administration of the drug.

Breaking of the blind was expressly forbidden except in the event of a medical emergency where the identity of the drug must be known in order to properly treat the subject, or in the event of an interim analysis, or in order to assess the dose escalation and stopping rules.

No unblinding occurred during study conduct.

Statistical methods

Analysis sets:

- **Randomized Set (RAN)** consisted of all subjects who were randomized to study treatment.
- **Safety analysis set (SAF)** consisted of all subjects who received a single-dose of the study drug. Subjects were classified according to treatment received.
- **Pharmacokinetic Analysis Set (PKAS)**: All subjects who complied sufficiently with the protocol, who received a single-dose of the study drug and had 1 pre-dose and at least 1 post-dose measurement of any of the PK assessment not impacted by any protocol deviations.

Blood samples not collected at the scheduled time were documented as sampling deviations. The actual time of collection of each blood sample was to be used for pharmacokinetic, pharmacodynamics, immunogenicity and statistical analysis.

Primary Endpoint Analysis (PK):

Analyses of variance (ANOVA) was to be performed on the natural log (ln)-transformed Cmax, AUC0-t and AUC0-inf PK parameters, with treatment as a fixed effect. 90% confidence intervals (CIs) for the geometric mean ratios (GMRs), ENZ215 (test)/EU-Prolia and ENZ215 (test)/US-Prolia were to be derived by

exponentiation of the CIs obtained for the difference between treatment least-squares means (LSM) resulting from the analyses on the ln-transformed PK parameters.

Bioequivalence criteria were to be considered met if the 90% CIs for the GMRs of Cmax, AUC0-t and AUC0-inf of denosumab for the Test vs. Reference Treatments (US-Prolia and EU-Prolia) fell within the limit of 80.00% to 125.00%.

Secondary Endpoint Analysis (PK):

The partial area under the curve pAUC0-28 days was to be compared between ENZ215 and Prolia using t-tests after log-transformation. The Wilcoxon-rank sum test was planned to be used for the comparison of tmax between ENZ215 and Prolia.

Pharmacokinetic exclusion criteria:

- No value for AUC0-inf, CL/F or T½ was to be reported for cases that do not exhibit a terminal log-linear phase in the concentration-time profile.
- Criteria for exclusion of pharmacokinetic parameters of a particular subject were as below:
    - Three consecutive missing (M) / Non-Reportable (NR) samples in *elimination* phase may significantly influence the AUC0-t, AUC0 - Day 28 and elimination phase dependent parameters (AUC0-inf and T1/2, CL/F). Inclusion of such parameters in the statistical analysis may mislead the final outcome. Hence, AUC0-t, AUC0 - Day 28 and elimination phase dependent parameters (AUC0-inf and T1/2) were to be excluded.
    - Additionally any subject with at least 3 consecutive Missing (M) / Non-reportable (NR) samples during the *absorption* phase such subject were to be excluded from the pharmacokinetic and statistical analysis. In such a scenario, only plasma concentration versus time data of that subject was to be tabulated and reported in the study report.
    - Subjects without measurable concentrations or who have only very low serum concentrations from the reference medicinal product were to be excluded from the pharmacokinetic and statistical analyses for the assessment of bioequivalence. A subject was considered to have very low serum concentrations if his/her AUC was less than 5% of reference medicinal product geometric mean AUC, calculated without inclusion of data from the outlying subject.
- Handling of Subjects with Non-Zero Pre-dose Concentrations:
  If non-zero pre-dose concentrations occur, the following procedure was to be used:
    - If the pre-dose concentration was less than or equal to 5% of the corresponding Cmax value for that subject, the subject's data was to be included in all PK measurements and calculations without any adjustment.
    - If the pre-dose value was greater than 5% of the corresponding Cmax value for that subject, the subject's data for the period in question was to be excluded from the statistical evaluations (descriptive statistics for that period and ANOVA).

Secondary Endpoint Analysis (Immunogenicity):

All immunogenicity data summaries were to be based on the safety analysis population. The frequency and percentage of positive ADA or NAb result was to be provided. The proportion of positive ADA or NAb in each treatment group was to be compared using chi-square or Fisher's exact tests. The p-value, relative risk, and corresponding 95% CI was to be presented.

Secondary Endpoint Analysis (Safety):

All outputs for safety outcomes were to be based on the SAF analysis set. There were no statistical comparisons between the treatment groups for safety data, unless otherwise specified with the relevant section.

Conduct of study

Study initiation date: 10 January 2022 (first subject signed informed consent)

Study completion date: 22 May 2024 (last subject last visit)

Database lock: 01 Jul 2024

The original protocol dated 07 October 2021 was amended 3 times.

Brief summaries of the non-administrative changes (made after the start of subject recruitment) are outlined below:

17 Mar 2022

- BMI range was modified in order to increase the subject recruitment (from ≥ 18.50 and ≤ 29.00 kg/m2 at the time of screening to ≥ 18.50 and ≤ 30.00 kg/m2).

11 Apr 2022

- Non-smokers criteria were deleted from Inclusion criteria (to include smokers consuming <10 cigarettes/day)
- Updated exclusion criteria number 17 and 20 and deleted number 15.
    - #15: Subject with a history of recurrent or chronic infections
    - #17: Received live vaccines within 4 weeks or last dose of COVID-19 vaccine within 14 days before screening or who may require live vaccine(s) during the study duration
    - #20: Subjects with suspected signs and symptoms of COVID-19/confirmed novel coronavirus infection (COVID-19) or with recent history (within 14 days) of travel/contact with any COVID-19 positive subject/isolation/quarantine.
      (instead of only confirmed novel COVID-19 infections)
- Updated study visit schedule for PK parameters and study procedure for COVID-19 subjects.
- Modified Urine analysis procedure including urine screen for cotinine test to check the compliance with respect to smoking restrictions.

03 Oct 2022

- Added EUDRACT number
- Modified exclusion criteria number 2, 5, 18, and 19 for better clarity.
    - #2: Participating or has received any investigational drug (or is currently using an investigational device) within 30 days before receiving the study drug, or at least 10 times the respective elimination halflife (whichever period is longer) *
      * For monoclonal antibody refer exclusion criteria number 18 and 19

- #5: Smokers who smoke ≥ 10 cigarettes or equivalent per day within 90 days prior to screening
- #18: Prior use of denosumab; and
  #19: Have previously been exposed to a monoclonal antibody or fusion protein within 270 days (other than denosumab) prior to randomisation and/or there is confirmed evidence or clinical suspicion of immunogenicity from previous exposure to a monoclonal antibody or fusion protein.
  (instead of: "Use of any monoclonal antibody including denosumab for a medical condition or in the context of another clinical study ")

- Administrative changes made for better protocol compliance in study visit schedule, changes in post-dose sampling, data capture methods, and CRFs/electronic CRFs.
- Clarified text related to unused investigational products as per the regulatory requirements, ethical considerations, precautions during COVID-19 Pandemic, SAEs, PK, PD, and safety population.
- Modified amount of blood for screening visit is increased from 10 mL to 15 mL to cover sufficient amount of blood for screening investigation.
- Total volume of blood withdrawn for the study was modified.
- Added text for pharmacokinetic exclusion criteria for better clarity.

The SAP was finalized on 21 Jun 2024. It was reported that no changes were made to the planned analyses after the finalization of the SAP.

Protocol Deviations and Quality Tolerance Limits

Deviations occurred in ALK22/ENZ215-DEN1

Protocol deviations were categorized as 'major' and 'minor' as follows:

- Major protocol deviation: A deviation from protocol-related procedures that could affect integrity of the data or adversely affect subjects. Such deviations require timely action.
- Minor protocol deviation: A deviation from accepted procedures that will not adversely affect subjects or data integrity, but should be dealt with appropriately.

Table 3. Protocol Deviations (Safety Analysis Set)

	Protocol Deviation Category	ENZ215 (N=68)	US-Prolia (N=69)	EU-Prolia (N=69)	All Subjects (N=206)
Subjects with protocol deviation		35 (51.5)	41 (59.4)	40 (58.0)	116 (56.3)
Informed Consent	Major	7 (10.3)	7 (10.1)	8 (11.6)	22 (10.7)
Laboratory Assessment	Major	9 (13.2)	9 (13.0)	9 (13.0)	27 (13.1)
Laboratory Assessment	Minor	12 (17.6)	15 (21.7)	17 (24.6)	44 (21.4)
Study Documentation	Minor	9 (13.2)	9 (13.0)	9 (13.0)	27 (13.1)
Study Procedures	Major	0	1 (1.4)	0	1 (0.5)
Study Procedures	Minor	28 (41.2)	30 (43.5)	32 (46.4)	90 (43.7)
Visit Schedule	Major	1 (1.5)	0	0	1 (0.5)
Visit Schedule	Minor	8 (11.8)	12 (17.4)	12 (17.4)	32 (15.5)

In total, 193 "minor" and 51 "major" deviations occurred in the DEN1 study. 116 subjects had minor deviations and 28 subjects out of them had "major" deviations (10 subjects in ENZ215 group and 9 subjects each, in EU-sourced Prolia and US-sourced Prolia groups).

All the major deviations were procedure related which included informed consent form (ICF) related, missing visits, additional cotinine test was not performed, and COVID-19 vaccination timing and CAPA for the same was appropriately filed.

Participant flow and numbers analysed

Of the 344 subjects screened for the study, 207 were randomized (68 subjects to ENZ215 group, 69 to EU-sourced Prolia group, and 70 to US-sourced Prolia group).

Table 4. Subject Disposition and Populations (Randomized Set)

Status		**ENZ215**	**US-Prolia**	**EU-Prolia**	**All Subjects**
Number of Subjects					
Randomized	N	68	70	69	207
Dosed	n (%)	68 (100.0)	69 (98.6)	69 (100.0)	206 (99.5)
Completed Study	n (%)	66 (97.1)	67 (95.7)	67 (97.1)	200 (96.6)
Prematurely Withdrawn	n (%)	2 (2.9)	3 (4.3)	2 (2.9)	7 (3.4)
Lost to Follow-up	n (%)	1 (50.0)	1 (33.3)	2 (100.0)	4 (57.1)
Other	n (%)	1 (50.0)	0	0	1 (14.3)
Personal Reason[a]	n (%)	0	2 (66.7)	0	2 (28.6)
Safety Analysis Set	n (%)	68 (100.0)	69 (98.6)	69 (100.0)	206 (99.5)
Pharmacokinetic Analysis Set	n (%)	68 (100.0)	69 (98.6)	69 (100.0)	206 (99.5)
Pharmacodynamic Analysis Set	n (%)	68 (100.0)	69 (98.6)	69 (100.0)	206 (99.5)

Note(s): Percentages for the reasons for discontinuation are based on the number of subjects who prematurely withdrew from the study. Percentages for dosed is based on the number of subjects randomized. All other percentages are based on the number of subjects dosed.

[a]: To be read and understood as 'withdrawal by subject'.

All randomized subjects received study treatment except 1 (in US-sourced Prolia group) who had withdrawn consent after randomization. The majority of screen failures were due to subjects not fulfilling the eligibility criteria. The most frequent reason for screen failure was subjects not meeting inclusion criterion number 3 (ie, BMI criteria), inclusion criterion number 9 (ie, willing and able to comply with the protocol requirements) and meeting exclusion criterion number 20 (ie, any reason/condition which would preclude subject's participation in the study as per the Investigator's opinion or warnings and contraindications in the prescribing information of Prolia).

Demographics and other baseline characteristics

Table 5. Demographics and Baseline Characteristics (Randomized set)

Variable/ Category	Statistic	ENZ215 (N=68)	US-Prolia (N=70)	EU-Prolia (N=69)	All Subjects (N=207)
Sex					
Male	n (%)	68 (100.0)	70 (100.0)	69 (100.0)	207 (100.0)
Age (years)					
	n	68	70	69	207
	Mean ± SD	37.9 ± 8.1	40.1 ± 7.8	38.9 ± 7.7	39.0 ± 7.9
	CV%	21.5	19.5	19.7	20.2
	Median	37.5	41.0	39.0	39.0
	Minimum,	28,	28,	28,	28,
	Maximum	55	54	55	55
Race					
Asian	n (%)	1 (1.5)	0	0	1 (0.5)
White	n (%)	67 (98.5)	70 (100.0)	69 (100.0)	206 (99.5)
Ethnicity					
Not Hispanic or Latino	n (%)	68 (100.0)	70 (100.0)	69 (100.0)	207 (100.0)
Height (cm)					
	n	68	70	69	207
	Mean ± SD	178.39 ± 6.22	178.41 ± 7.69	178.96 ± 6.44	178.59 ± 6.79
	CV%	3.5	4.3	3.6	3.8
	Median	179.00	180.00	179.00	179.00
	Minimum,	166.0,	157.0,	163.0,	157.0,
	Maximum	194.0	193.0	195.0	195.0
Weight (kg)					
	n	68	70	69	207
	Mean ± SD	83.62 ± 10.75	81.56 ± 11.67	84.16 ± 10.34	83.10 ± 10.94
	CV%	12.9	14.3	12.3	13.2
	Median	84.00	82.40	85.00	83.80
	Minimum,	56.5,	55.5,	58.4,	55.5,
	Maximum	112.6	107.0	102.8	112.6
BMI (kg/m^2)					
	n	68	70	69	207
	Mean ± SD	26.219 ± 2.601	25.562 ± 2.835	26.260 ± 2.760	26.010 ± 2.740
	CV%	9.9	11.1	10.5	10.5
	Median	26.220	25.925	26.540	26.160
	Minimum,	20.26,	19.51,	18.64,	18.64,
	Maximum	29.95	29.95	29.98	29.98

Note(s): Abbreviations: BMI = body mass index; CV% = coefficient of variation; EU = European Union; SD = standard deviation; US = Unites States.

Outcomes

Primary PK endpoints

Table 6. Bioequivalence Assessment (Pharmacokinetic Analysis set)

Treatment Comparison Statistics	**C_{max}**	**$AUC_{(0\text{-}inf)}$**	**$AUC_{(0\text{-}t)}$**	**Bioequivalence Criteria (Yes/No) ***
ENZ215 (N=68) Vs EU sourced Prolia (N=69)				
Geometric LS Means (CV%)				Yes
ENZ215	7662.99 (32.6)	7622698.42 (36.4)	7508134.18 (36.8)	
EU sourced Prolia	7742.64 (23.2)	7779487.60 (24.7)	7674912.81 (24.9)	
Inter-Subject CV (%)	28.2	31.0	31.3	
(%) Ratio [90% CI]	98.97 [91.52, 107.03]	97.98 [89.82, 106.89]	97.83 [89.60, 106.81]	
ENZ215 (N=68) Vs US sourced Prolia (N=69)				
Geometric LS Means (CV%)				Yes
ENZ215	7662.99 (32.6)	7622698.42 (36.4)	7508134.18 (36.8)	
US sourced Prolia	8033.14 (24.9)	7934699.65 (28.9)	7842297.13 (28.8)	
Inter-Subject CV (%)	28.9	32.8	33.0	
(%) Ratio [90% CI]	95.39 [88.04, 103.36]	96.07 [87.63, 105.31]	95.74 [87.29, 105.01]	

Abbreviations: N = number of subjects; CV% = coefficient of variation; CI=Confidence Interval.
Note: *Yes, if the 90% CI for geometric LSM ratios of ln-transformed parameters $AUC_{(0\text{-}inf)}$, $AUC_{(0\text{-}t)}$ and C_{max} and falls within the acceptance range of 80.00% to 125.00% else No.;
The treatment will be a fixed effect in the ANOVA model.

Serum Denosumab Pharmacokinetic Parameters

Table 7. Summary of Serum Denosumab Pharmacokinetic Parameters by Treatment (Pharmacokinetic Population)

Treatment Statistic	$AUC_{(0\text{-}inf)}$ (h*ng/mL)	$AUC_{(0\text{-}t)}$ (h*ng/mL)	C_{max} (ng/mL)	$AUC_{(0\text{-}Day28)}$ (h*ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (mL/h)
ENZ215, (N=68)							
n	66	66	68	66	68	66	66
Mean	8060737.96	7950718.07	7995.29	3734634.99		467.496	0.008
SD	2641068.44	2647150.54	2122.72	891887.69		124.138	0.004
CV%	32.8	33.3	26.5	23.9		26.6	51.0
Minimum	1590552.7	1565099.4	1760.0	990834.4	47.22	208.30	0.00
Median	7568790.8	7421891.3	8190.0	3735741.8	120.62	459.58	0.01
Maximum	17460831.4	17424914.0	14200.0	5971879.2	360.63	716.26	0.04
Geo mean	7622698.42	7508134.18	7662.99	3608894.44		450.745	0.008
GCV%	36.4	36.8	32.6	28.9		28.3	36.4
EU sourced Prolia®, (N=69)							
n	67	67	69	67	69	67	67
Mean	8008973.81	7904876.44	7930.87	3794350.13		461.489	0.008
SD	1971572.75	1959500.24	1648.45	720450.22		100.202	0.002
CV%	24.6	24.8	20.8	19.0		21.7	24.8
Minimum	4038476.7	3975053.3	4000.0	2085037.1	46.93	260.99	0.00
Median	7695957.7	7533810.9	8070.0	3838268.5	120.08	458.69	0.01
Maximum	14378509.0	14248605.1	11600.0	5260627.5	359.75	669.04	0.01
Geo mean	7779487.60	7674912.81	7742.64	3721138.61		450.207	0.008
GCV%	24.7	24.9	23.2	20.6		23.2	24.7
US sourced Prolia®, (N=69)							
n	67	67	69	67	69	67	67
Mean	8248514.90	8151301.43	8267.83	3886040.36		456.301	0.008
SD	2322466.47	2291979.13	1975.98	826633.65		116.159	0.002
CV%	28.2	28.1	23.9	21.3		25.5	29.8
Minimum	4056652.4	4048307.1	3890.0	1973422.2	46.98	250.28	0.00
Median	8337331.3	8258310.7	8180.0	3879146.4	120.28	451.92	0.01
Maximum	16337643.8	16068408.3	13700.0	6007931.9	479.92	795.28	0.01
Geo mean	7934699.65	7842297.13	8033.14	3796048.41		441.262	0.008
GCV%	28.9	28.8	24.9	22.5		27.1	28.9

Abbreviations: CV% = coefficient of variation; EU = European Union; GCV% = geometric CV%; Geo = geometric; N = number of subjects; n = number of available subjects; SD = standard deviation; US = United States.
Note(s): For t_{max}, only n, median, minimum, and maximum were presented.
Source: Table 14.2.1.2.

Statistical Issues

One subject was early terminated. The subject visited the site to perform the end of study visit assessments, per protocol described for Visit 20 (Day 270/EOS) (6456 H). Samples PK, CTX-1 and ADA/NAb were collected per protocol and labeled with the fixed label for Visit 20. This led to the laboratory raw data to label such samples as "Day 270".

For statistical analysis, the PK sample was associated to Visit 13 (Day 42) (984 H), since the subject was early terminated on the matching day of his pre-planned Day 42 visit. However, for CTX-1 and ADA/NAb these assessments were unscheduled and thus the same was not part of any summary table for the protocol defined visits but the same was part of the listing

Serum Denosumab Concentrations

Figure 2. *Mean ± SD Denosumab Serum Concentration-time Profiles by Scheduled Time on Linear and Semi-logarithmic Scales (Pharmacokinetic Analysis Set)*

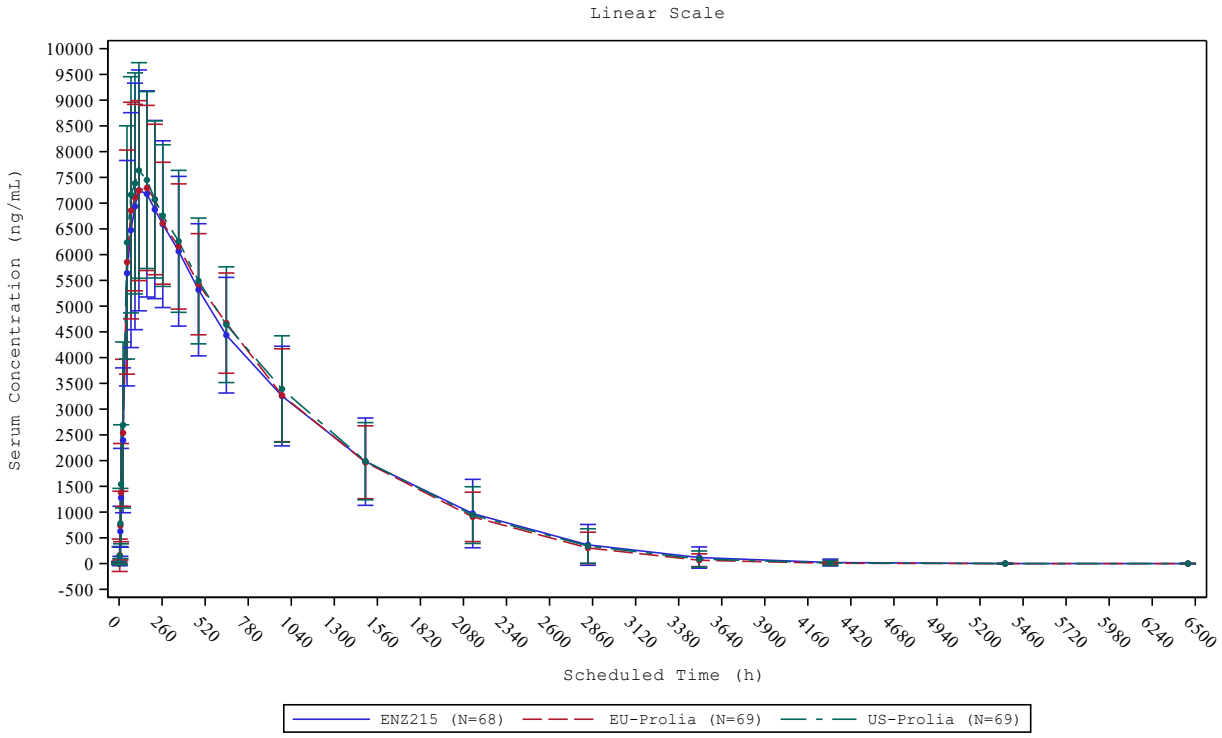
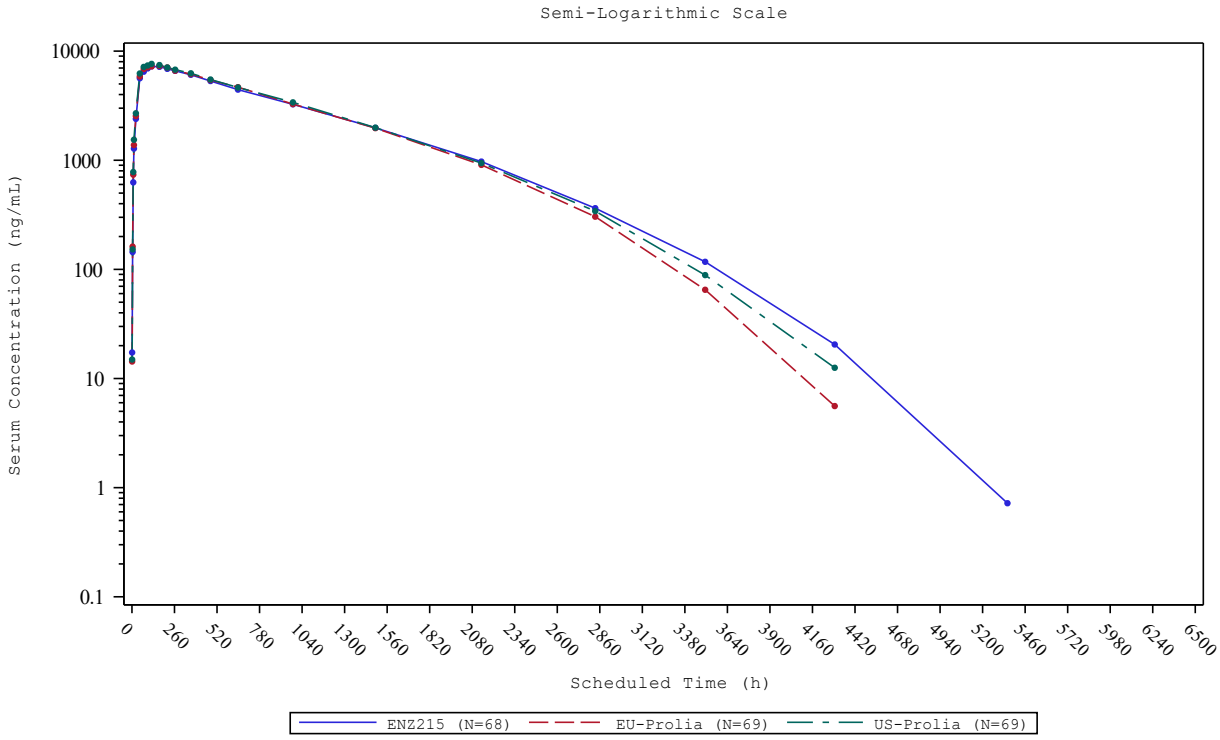


Figure: Mean ± SD Denosumab Serum Concentration-time Profiles by Scheduled Time on Linear and Semi-logarithmic Scales (Pharmacokinetic Analysis Set)

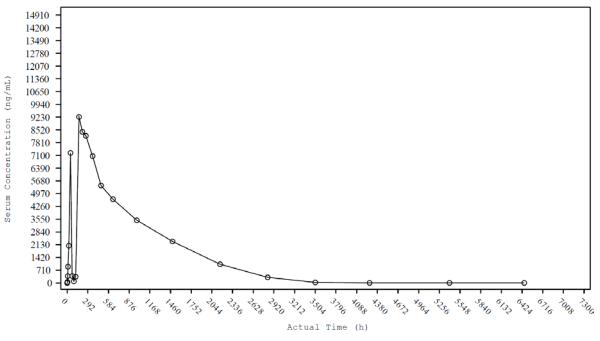


Individual Denosumab Serum Concentration-time Profiles

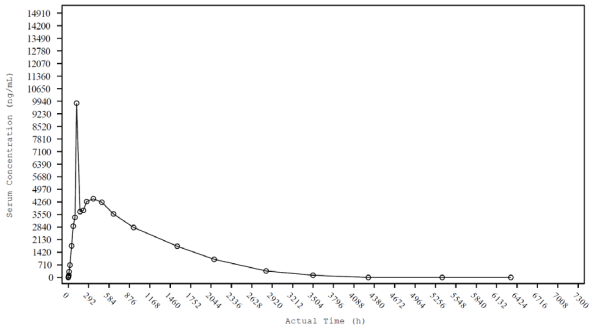
Individual denosumab serum concentrations vs time profiles were provided for all subjects. Below, sample serum concentration-time profiles shows spikes in the PK profile.

Figures: Individual Denosumab Serum Concentration-time Profiles on Linear Scale (Pharmacokinetic Analysis Set)

Treatment/Participant ID: ENZ215/02-042 (drop occurred between 72-120h according to Listing 16.2.6.2)

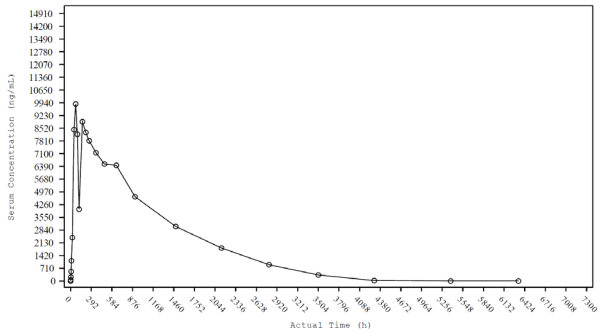


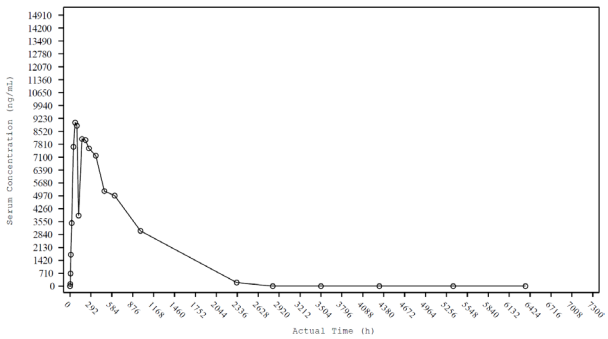
Treatment/Participant ID: ENZ215/03-016 (peak at 120h)



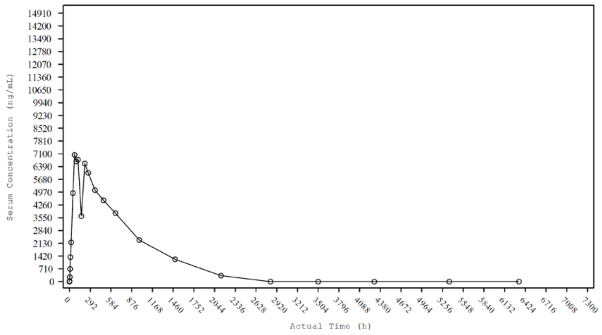
Treatment/Participant ID: ENZ215/03-025 (drop at 120h)

Treatment/Participant ID: EU-Prolia/02-125 (drop at 120h)





Treatment/Participant ID: EU-Prolia/03-013
(drop at 168h)



2.5.2.1.2. ALK22/ENZ215-DEN2 (Pharmacokinetics in the target population)

Study ALK22/ENZ215-DEN2 was a Phase 3, randomized, double-blind, parallel-group, active-controlled study to compare the efficacy, safety, PD, PK, and immunogenicity of ENZ215 and Prolia in 504 postmenopausal women with osteoporosis aged ≥ 55 and ≤ 85 years. The study was divided into three periods: Screening period: up to 35 days; Double-blind treatment period of 12 months; and Open-label, switch-over period of six months. The 60 mg dose of either ENZ215 or Prolia were administered subcutaneously in upper thigh on Day 1 and Month 6 for all participants during the double treatment period and on Month 12 for participants in the open-label extension period.

For more details about the study design please refer to the efficacy section. The PK results of this study are presented below.

PK assessments

PK parameters (Cmax, Tmax, AUC(0-1M) and AUC(0-6M)) of denosumab were measured at baseline (Day 1), Day 8, Day 15, Month 1, Months 3, and Month 6 (prior to second dose). Ctrough was measured at Month 12 (i.e., 6 months after the second dose). No PK measurements were scheduled for the open-label extension phase beyond Month 12. For PK sampling on Day 8 and Day 15, the visit window was ±3 days but a window period of ±2 hours was allowed in relation to the time of IP administration on Day 1.

Statistical methods

The pharmacokinetic (PK) set was defined to include all randomized patients who received at least one dose of study intervention, with at least one valuable PK endpoint (Cmax or AUC0-6 month) and no major protocol deviations affecting the PK parameters up to Month 12. All the protocol deviations (irrespective of major / minor PDs) with action for analysis that leads to exclusion from PK Set were to be considered for exclusion from PK Set in the PK analysis set derivation.

PK parameters were to be calculated by NCA methods from the concentration-time data using Phoenix WinNonlin Version <8.3> or higher following these guidelines:

- Actual sampling times relative to dosing were to be used in the calculation of all derived pharmacokinetic parameters.
- No imputation of missing data was planned.

Handling of BLQ samples for derivation of serum PK parameters after single dose administration

- BLQs at the beginning of a subject profile (i.e., before the first incidence of a measurable concentration) were to be assigned to zero.
- BLQs at the end of a subject profile (i.e., after the last incidence of a measurable concentration) were to be set to zero.
- Single BLQs which fell between two measurable concentrations were to be set to missing.
- Consecutive BLQs which fell between measurable concentrations were to be set to missing.
- Measurable concentrations after consecutive BLQs also were to be set to missing.

Handling of BLQ samples for derivation of plasma PK parameters after multiple dose administration

- BLQs for Day 1 at the beginning of a participant profile (i.e., before the first incidence of a measurable concentration) were to be assigned to zero.
- BLQs on subsequent dosing days and not separated by a washout: pre-dose values, BLQs in the absorption phase, and BLQs between evaluable concentrations, were tol be substituted by zero before the calculation of the PK variables.
- Terminal BLQs (at the end of participant profile) were tol be set to zero.

Pharmacokinetic parameters will be estimated according to the following rules:

Table 8. Pharmacokinetic Parameter and Estimation

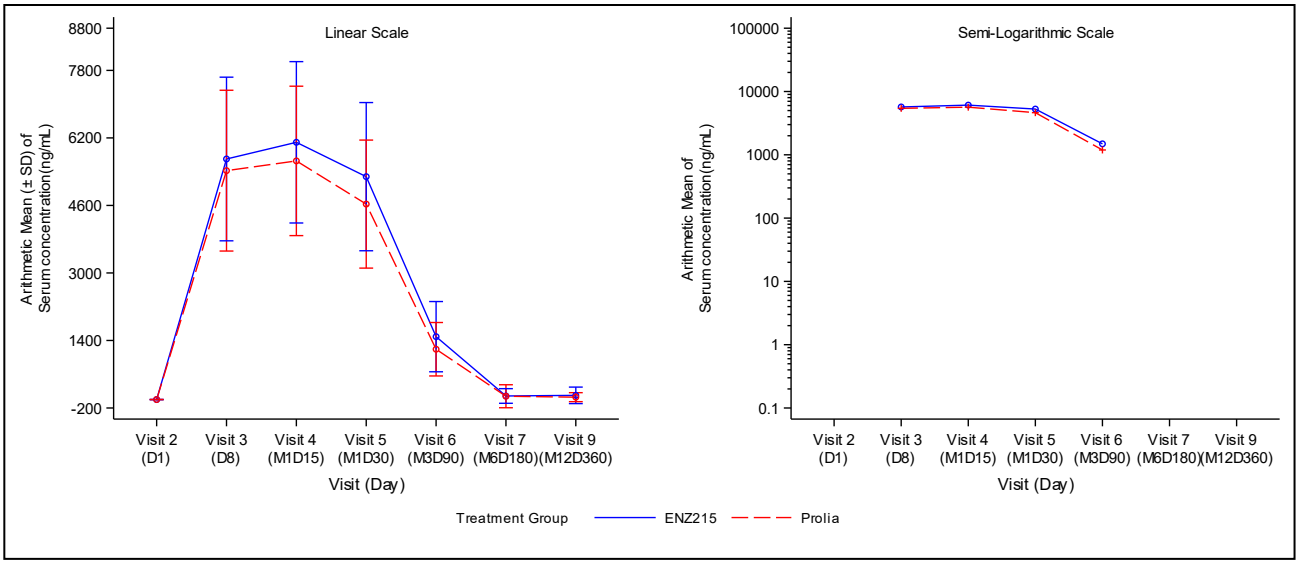
Parameter	Guideline for Derivation
C_{max}, t_{max}, C_{trough},	Obtained directly from the observed concentration-time data
AUC_{0-x}	The AUC from zero time (pre-dose) to the time of specific time x will be calculated by a combination of linear and logarithmic trapezoidal methods. Unless specifically requested and justified, the linear up/log down trapezoidal method will be employed. The AUC from zero time to the specific time x is the sum of areas up to the specific time x sample: $AUC_{0-x}=AUC_{0-x} = \int_0^x Cx * dx$

The PK parameters were to be analysed descriptively.

Outcomes

The PK set included 57 patients (22.5% of patients from the safety set) in ENZ215 group and 59 patients (23.5%) in Prolia group.

Figure 3. Arithmetic Mean (± SD) of ENZ215 and Prolia Serum Concentration-Nominal Time Data (Linear Scale and Semi-Logarithmic Scale) (PK Set)



Abbreviations: PK = pharmacokinetics; SD = standard deviation.

Table 9. Summary Statistics of ENZ215 and Prolia Pharmacokinetics Parameter by Treatment (PK Set)

Parameter (unit)	Statistics	ENZ215 (N = 57)	Prolia (N = 59)
C_{max} (ng/mL)	n	57	59
	Arithmetic Mean (CV%)	6400 (31.3)	6010 (30.6)
	Geometric Mean (geoCV%)	6000 (41.5)	5720 (32.9)

Parameter (unit)	Statistics	ENZ215 (N = 57)	Prolia (N = 59)
	Minimum	1500	2790
	Median	6610	5890
	Maximum	9640	9760
T_{max} (h)	n	57	59
	Minimum	120.05	118.77
	Median	335.22	328.53
	Maximum	719.48	695.97
AUC_{0-1M} (h*ng/mL)	n	56[a]	57[b]
	Arithmetic Mean (CV%)	3420000 (30.1)	3160000 (32.3)
	Geometric Mean (geoCV%)	3220000 (40.4)	3000000 (33.1)
	Minimum	876000	1570000
	Median	3610000	3090000
	Maximum	4990000	6230000
AUC_{0-3M} (h*ng/mL)	n	54[c]	57[b]
	Arithmetic Mean (CV%)	7840000 (33.9)	6820000 (33.7)
	Geometric Mean (geoCV%)	7260000 (45.7)	6450000 (35.2)
	Minimum	1820000	2880000
	Median	8220000	6750000
	Maximum	13200000	12700000
AUC_{0-6M} (h*ng/mL)	n	54[d]	57[b]
	Arithmetic Mean (CV%)	9160000 (35.8)	7930000 (35.3)
	Geometric Mean (geoCV%)	8420000 (48.0)	7460000 (36.7)
	Minimum	2050000	3120000
	Median	9540000	7640000
	Maximum	16700000	15700000
AUC_{6M-12M} (h*ng/mL)	n	29[e]	23[f]
	Arithmetic Mean (CV%)	696000 (129.4)	489000 (110.9)
	Geometric Mean (geoCV%)	312000 (232.1)	301000 (130.5)
	Minimum	41800	68000
	Median	291000	263000
	Maximum	4010000	2290000

Abbreviations: N = The total number of patients in PK Set; n = Number of patients with non-missing data within the specific Category; PK = Pharmacokinetics; SD = Standard Deviation; CV = Coefficient of Variation.
Geometric CV% = SQRT (es2-1)*100, where s is the SD of the log transformed values.
a: 1 participant excluded; b: 2 participants excluded; c: 3 participants excluded; d: 3 participants excluded; e: 28 participants excluded; 36 participants excluded.

Table 10. Summary Statistics of ENZ215 and Prolia C_{trough} by Treatment (PK Set)

Parameter (unit)	Statistics	ENZ215 (N = 57)	Prolia (N = 59)
$C_{trough\ 6M}$ (ng/mL)	n	54[a]	57[b]
	Arithmetic Mean (CV%)	84.7 (204.7)	78.2 (348.6)
	Geometric Mean (geoCV%)	114 (173.0)	122 (149.1)
	Minimum	0.00	0.00
	Median	0.00	0.00
	Maximum	837	1960
$C_{trough\ 12M}$ (ng/mL)	n	52[c]	53[d]
	Arithmetic Mean (CV%)	99.4 (196.8)	57.4 (187.0)
	Geometric Mean (geoCV%)	120 (168.9)	95.0 (108.8)
	Minimum	0.00	0.00
	Median	0.00	0.00
	Maximum	1040	519

N = The total number of patients in PK Set; n = Number of patients with non-missing data within the specific Category.
PK = Pharmacokinetics; SD = Standard Deviation; CV = Coefficient of Variation;
Geometric CV% = SQRT(es2-1)*100, where s is the SD of the log transformed values.
a: 3 participants excluded; b: 2 participants excluded; c: 5 participants excluded; d: 6 participants excluded
Source: Source: CSR ALK22/ENZ215-DEN2 Table 11-19

For many participants, median denosumab $C_{trough\ 6M}$ and $C_{trough\ 6M}$ for the ENZ215 group and Prolia group were all 0.00 ng/mL due to *below the limit of quantification* records.

2.5.2.2. Pharmacodynamics

The pharmacodynamics of ENZ215 and Prolia have been investigated in two studies (ALK22/ENZ215-DEN1 [healthy adult men]; ALK22/ENZ215-DEN2 [post-menopausal women with osteoporosis]). Apart from these studies, no other clinical pharmacology studies (i.e., drug interaction studies, or studies in special populations such as hepatic or renal impairment) were performed.

Mechanism of action

Denosumab, is a human monoclonal antibody (IgG2) that targets and binds with high affinity and specificity to RANKL (receptor activator of nuclear factor kappa-β ligand), a transmembrane or soluble protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. Denosumab prevents RANKL from activating its receptor. Prevention of the RANKL/RANK interaction inhibits osteoclast formation, function, and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone. RANKL is an upstream regulator of pathways linked to a broad range of bone loss related conditions. Denosumab acts as a highly targeted agent that blocks RANKL and exerts its therapeutic effect across all approved bone loss conditions. It is expected that a product highly similar to Prolia in its binding properties to RANKL will exert the same pharmacological effect regardless of any other systemic or bone related factors. Therefore, once biosimilarity has been convincingly shown between the proposed biosimilar, ENZ215, and reference products, there is a high level of assurance that the clinical performance of the proposed biosimilar will be the same across indications associated with Prolia.

Bioanalytical methods

Determination of CTX1 and P1NP in Human Serum on COBAS system

The methods for the determination of the PD parameters CTX1 (C-terminal telopeptide) and P1NP (Procollagen-type 1 N-terminal-propeptide) in human serum are based on an electrochemiluminescence immunoassay (ECLIA) on a COBAS 8000 instrument. The assays were validated at Eurofins Central Laboratory Breda with respect to limits of quantification, accuracy, intra-/inter-assay precision, selectivity (drug/matrix interference), haemolysis/hyperlipidaemia, parallelism/dilution, and stability. The assays were well described and established, and the presented methods appear to be suitable for the determination of CTX1 and P1NP in human serum.

Primary and Secondary pharmacology

2.5.2.2.1. ALK22/ENZ215-DEN1

For a detailed assessment of the design of study ALK22/ENZ215-DEN1, please refer to section 2.5.2.1.1. Only PD specific aspects are provided below.

Bone Biomarkers

The bone turnover PD biomarker carboxy-terminal cross-linking telopeptide of type 1 collagen (CTX) was measured in the DEN1 study.

Statistical methods

sCTX:

Pharmacodynamic Analysis Set (PDAS): All subjects who complied sufficiently with the protocol, who received a single-dose of the study drug and had 1 pre-dose and at least 1 post-dose measurement of any of the PD assessment.

Blood samples not collected at the scheduled time were documented as sampling deviations. The actual time of collection of each blood sample was to be used for pharmacokinetic, pharmacodynamics, immunogenicity and statistical analysis.

Analysis of pharmacodynamic parameter

The AUEC was to be calculated as the area under the effect curve from baseline until CTX-1 values returned to baseline for the first time. An analysis of covariance was to be performed on the log-transformed AUEC, including treatment as a fixed effect and baseline CTX-1 value as covariate. The assessment of serum CTX-1 similarity as a secondary endpoint was to be based upon the 95% CIs for the ratio of the GMs (ENZ215 and Prolia) for AUEC of baseline-corrected serum CTX-1 (ie, percentage [%] change from baseline), which had to be contained entirely within the pre-specified limits of 0.80 to 1.25.

Pharmacodynamic exclusion criteria:

Criteria for exclusion of pharmacodynamics parameter of a particular subject were as below:

- Three consecutive missing(m) samples in the late phase may significantly influence AUEC, such subjects were not to be considered for the AUEC comparison.

- Subjects having any major protocol deviations or other clinical observations that can impact the PD.

For PD parameter calculations, predose samples that are BLQ were to be assigned a numerical value of zero. BLQ values observed during the reduction of the profile were to be set to zero. BLQ values embedded between 2 quantifiable data points were to be set to missing when calculating PD parameters. Actual elapsed time from dosing was to be used if a sample was collected outside the time window period otherwise scheduled time was to be used for the final serum PD parameter calculations.

Outcomes

sCTX:

Secondary PD endpoint: Area under the effect curve (AUEC) from time 0 to Day 270 for serum CTX-1 percent inhibition

Table 11. Pharmacodynamic Assessment (Pharmacodynamic Analysis set)

Treatment Comparison Statistics	AUEC
ENZ215 (N=68) Vs EU sourced Prolia (N=69)	
Geometric LS Means (CV%)	
ENZ215	432174.780 (20.1)
EU sourced Prolia	442806.204 (13.6)
Ratio [95% CI]	0.98 [0.92, 1.03]
ENZ215 (N=68) Vs US sourced Prolia (N=69)	
Geometric LS Means (CV%)	
ENZ215	430341.820 (20.1)
US sourced Prolia	436622.000 (18.6)
Ratio [95% CI]	0.99 [0.93, 1.05]

Abbreviations: N = number of subjects; CV% = coefficient of variation; CI=Confidence Interval. ANCOVA model will be used including treatment as a fixed effect and baseline CTX-1 value as covariate.
Source: CSR study ALK22/ENZ215-DEN1, Table 7

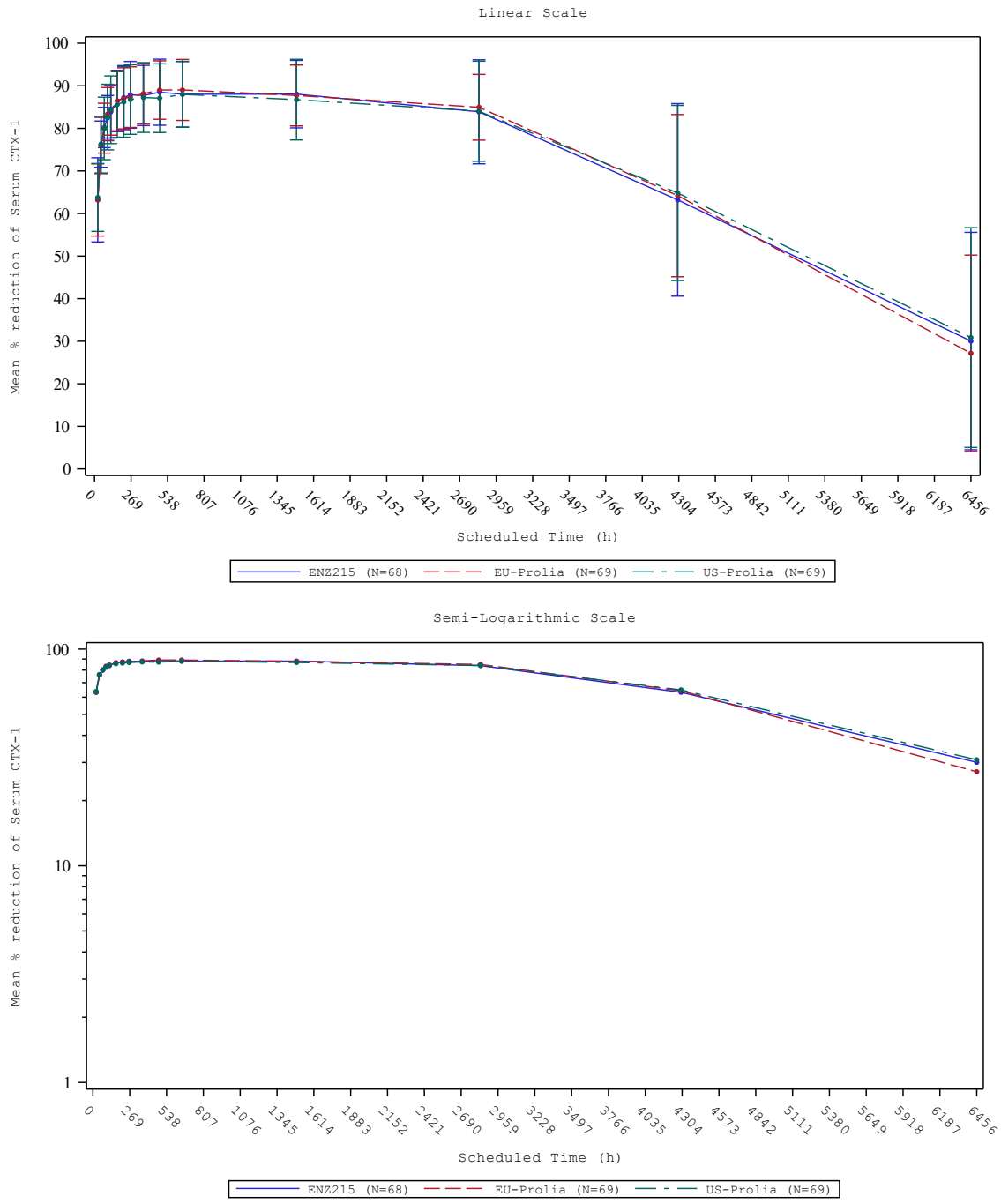
Table 12. Summary of CTX-1 Serum Pharmacodynamic Parameters (Pharmacodynamic Analysis set)

Treatment Statistic	ENZ215, (N=68) AUEC (h*%)	EU-Prolia, (N=69) AUEC (h*%)	US-Prolia, (N=69) AUEC (h*%)
	n=58	n=61	n=63
Mean	437624.9301	448700.5470	443877.5472
SD	76859.8471	53961.9945	74084.7842
CV%	17.6	12.0	16.7
Minimum	190520.213	236864.343	232245.095
Median	444846.632	453019.670	465352.399
Maximum	568379.429	554060.546	549021.063
Geo mean	429927.2277	445006.9559	437009.6173
GCV%	20.1	13.6	18.6

Abbreviations: CV% = coefficient of variation; GCV% = geometric CV%; Geo = geometric; N = number of subjects; n = number of available subjects; ND = not determined; SD = standard deviation.
Source: CSR study ALK22/ENZ215-DEN1, CSR Table 14.2.2.3

The mean AUEC values were relatively close, with ENZ215 at 437,624.9301 h%, EU sourced Prolia at 448,700.5470 h%, and US sourced Prolia at 443,877.5472 h%, with some differences in variability were observed (CV% ranging from 12.0% to 17.6%).

Figure 4. Mean reduction from baseline CTX-1 Serum Concentrations-time Profiles by Scheduled Time on Linear and Semi-logarithmic Scales (Pharmacodynamic Analysis Set)



Immediately following study treatment administration, mean percent change from baseline in CTX-1 showed a sharp decrease until 264 to 648 hours post dose, with low levels of CTX-1 persisting until 2832 hours post dose in all three groups, before gradually increasing. The largest reduction in CTX-1 was observed from 480

to 648 hours post dose, with mean percent change from baseline at −88.5%, −89.0% and −88.0% in ENZ215, EU-Prolia, and US- Prolia groups, respectively.

Statistical Issues

One subject was early terminated. The subject visited the site to perform the end of study visit assessments, per protocol described for Visit 20 (Day 270/EOS) (6456 H). Samples PK, CTX-1 and ADA/NAb were collected per protocol and labeled with the fixed label for Visit 20. This led to the laboratory raw data to label such samples as "Day 270".

For statistical analysis, the PK sample was associated to Visit 13 (Day 42) (984 H), since the subject was early terminated on the matching day of his pre-planned Day 42 visit. However, for CTX-1 and ADA/NAb these assessments were unscheduled and thus the same was not part of any summary table for the protocol defined visits but the same was part of the listing.

2.5.2.2.2. ALK22/ENZ215-DEN2 (main PD study)

Study ALK22/ENZ215-DEN2 investigated two co-primary endpoints:

- Efficacy: Percentage change in BMD at lumbar spine (L1-L4 region) measured by DXA from baseline to Month 12 (discussed in the efficacy section 3.3.4, including the study design)
- PD: AUEC of sCTX over the initial six months (from Day 1 pre-dose to Month 6 pre-dose) (discussed here)

Serum procollagen type I N-terminal propeptide (sP1NP) was analyzed as a secondary PD endpoint.

Statistical methods

sCTX (co-primary endpoint):

Percentage change from baseline %CfB in sCTX was to be computed as follows:

$$\%CfB = \frac{sCTX_{baseline} - sCTX_{timepoint}}{sCTX_{baseline}} \cdot 100,$$

where $sCTX_{baseline}$ and $sCTX_{timepoint}$ respectively are concentration value at baseline (pre-dose at Day 1) and at post-baseline timepoint.

The AUEC from zero time (pre-dose) to month 6 was to be calculated by a combination of linear-linear methods as the sum of areas up to month 6:

$$AUEC = \int_0^{6\,month} Cx * dx.$$

The PD set was defined to include all randomized patients who received at least one dose of study intervention, whose sCTX values were available in order to calculate PD parameter AUEC values for primary analysis and had no major protocol deviations which affected sCTX or sP1NP measurement.
The PD set was to be used as the primary analysis set for PD data.

Co-Primary Endpoints Analysis (PD):

To evaluate the AUEC of sCTX, the treatment comparison was to be made using an ANCOVA model on log-transformed data of AUEC of sCTX with treatment group and baseline sCTX value as a independent variables. The ANCOVA was to include calculation of LSM for the treatment groups. The ratios of LSM were calculated

using the exponentiation of the LSM from the analyses on the corresponding log-transformed AUEC of sCTX on the PD set. Pharmacodynamic equivalence was to be concluded if the 90% CI of the treatment ratio was contained within the acceptance limits of 80% to 125%. 95% CIs of geometric mean ratio between the treatment groups also were to be provided as an exploratory analysis.

In the primary PD analysis, patients with missing sCTX sampling at Baseline were not to be included in the analysis of the AUEC of sCTX. Additionally, to be included in the analysis of the AUEC of sCTX, patients needed to have the results from the visits of Day 1, Day 15, Day 30 (M1), and Day 180 (M6). Serum sCTX concentration below the limit of quantification (BLQ) were to be considered as BLQ in summary tables. sCTX values < BLQ were to be considered as zero (0) value in the analysis. sCTX values missing at baseline visit and sCTX value of zero (0) or < BLQ at baseline were not to be considered in the primary PD endpoint analysis nor in the summary statistics analysis.

Secondary Endpoints Analysis (Efficacy/PD):

The individual serum concentrations of sCTX and sP1NP were summarised by treatment group at each planned sampling time using descriptive statistics. Percent change from baseline in sP1NP was summarised in tabular and graphical format using the PD Set. A by-participant listing was provided for sCTX and sP1NP concentrations data.

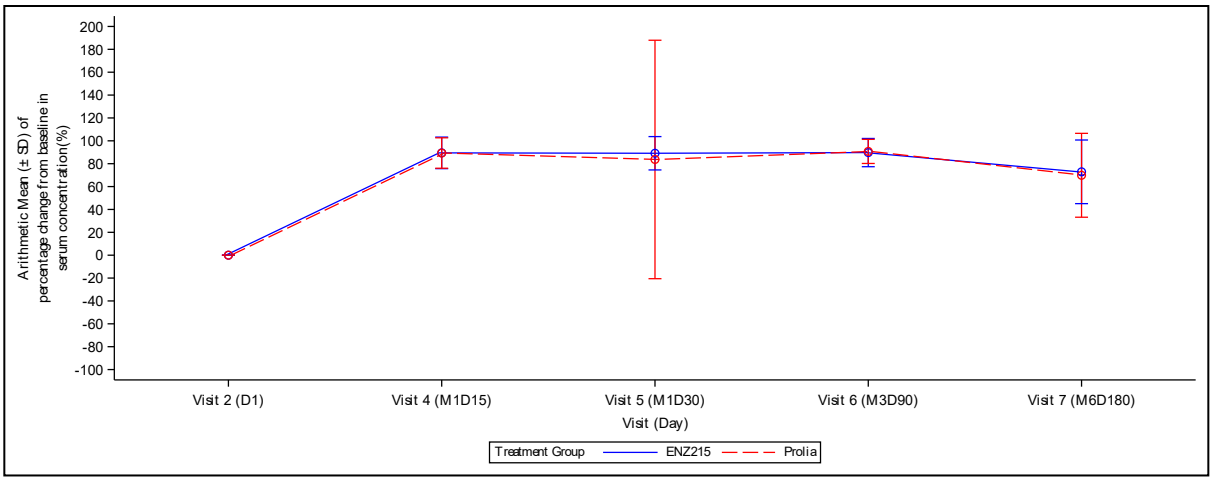
Outcomes

sCTX (co-primary endpoint):

AUEC of sCTX Over the Initial 6 Months (From Day 1 Pre-dose to Month 6 Pre-dose):

At baseline, the mean (SD) serum concentration of sCTX was 0.4897 (0.2081) ng/mL in the ENZ215 group and 0.4904 (0.2121) ng/mL in the Prolia group.

Figure 5. Arithmetic Mean (± SD) for percentage change from baseline in sCTX serum concentration of ENZ215 and Prolia groups- nominal time data -(linear scale) (PD set)



Abbreviations: PD = pharmacodynamics; sCTX = serum C-telopeptide of type 1 collagen; SD = standard deviation.
Source: CSR ALK22/ENZ215-DEN2 Figure 11-2

Table 13. Summary of observed and percent change from baseline in serum concentration of sCTX (ng/mL) by visit, treatment group (PD set)

		ENZ215 (N=234)				Prolia® (N=237)			
Visit/(Day)	Statistics	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline (%CfB)	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline (%CfB)
Baseline (D1)	n	234				237			
	Mean	0.4897				0.4904			
	SD	0.2081				0.2121			
	Minimum	0.048				0.045			
	Median	0.4690				0.4760			
	Maximum	1.080				1.130			
Visit 4 (M1D15)	n	234	234	234	234	237	237	237	237
	Mean	0.4897	0.0428	0.4469	89.4597	0.4904	0.0418	0.4486	89.3384
	SD	0.2081	0.0321	0.2019	13.7974	0.2121	0.0315	0.2083	13.3032
	Minimum	0.048	0.000	-0.024	-50.000	0.045	0.000	-0.004	-8.889
	Median	0.4690	0.0500	0.4205	90.8045	0.4760	0.0500	0.4360	90.8580
	Maximum	1.080	0.184	1.020	100.000	1.130	0.203	1.068	100.000
Visit 5 (M1D30)	n	234	234	234	234	237	237	237	237
	Mean	0.4897	0.0436	0.4461	89.1216	0.4904	0.0443	0.4461	83.6980
	SD	0.2081	0.0363	0.2047	14.5808	0.2121	0.0622	0.2221	104.2425
	Minimum	0.048	0.000	-0.018	-37.500	0.045	0.000	-0.768	-1505.882
	Median	0.4690	0.0500	0.4235	90.7950	0.4760	0.0490	0.4350	90.8200
	Maximum	1.080	0.257	1.031	100.000	1.130	0.819	1.090	100.000
Visit 6 (M3D90)	n	234	234	234	234	237	237	237	237
	Mean	0.4897	0.0414	0.4483	89.7139	0.4904	0.0389	0.4515	90.7335
	SD	0.2081	0.0329	0.2050	12.3470	0.2121	0.0313	0.2083	10.5377
	Minimum	0.048	0.000	-0.007	-14.583	0.045	0.000	0.007	13.725
	Median	0.4690	0.0490	0.4295	90.7230	0.4760	0.0500	0.4460	91.2030
	Maximum	1.080	0.220	1.017	100.000	1.130	0.125	1.090	100.000
Visit 7 (M6D180)	n	234	234	234	234	237	237	237	237
	Mean	0.4897	0.1096	0.3801	72.8928	0.4904	0.1165	0.3739	69.8648
	SD	0.2081	0.0763	0.2102	27.8172	0.2121	0.0729	0.2109	36.6679
	Minimum	0.048	0.000	-0.151	-231.250	0.045	0.000	-0.167	-327.451
	Median	0.4690	0.0945	0.3645	79.6595	0.4760	0.0970	0.3730	77.8150
	Maximum	1.080	0.683	0.957	100.000	1.130	0.442	1.030	100.000

N = The total number of patients in PD Set; n = Number of patients with non-missing data within the specific category; PD = Pharmacodynamic.

SD = Standard Deviation; sCTX = Serum C-Telopeptide of Type 1 Collagen.

%CfB in serum CTX will be computed as, %CfB = (CTXbaseline - CTXtimepoint/CTXbaseline)*100.

Source: Listing 16.2.8.4.

Table 14. Analysis of Covariance of AUEC of Percentage Change in sCTX From Baseline to Month 6 (PD Set)

Treatment Group	n	Geometric Mean	95% CI	Ratio of Geometric Means	90% CI of Geometric Mean Ratio	95% CI of Geometric Mean Ratio
ENZ215 (N = 234)	233	347382.787	340087.2488, 354834.8289	1.004	0.9795, 1.0299	0.9748, 1.0349
Prolia (N = 237)	236	345858.423	338640.7259, 353229.9557			

Abbreviations: ANCOVA = analysis of covariance; AUEC = area under the effect curve; CI = confidence interval; N = total number of participants in PD Set; n = number of participants with non-missing data within the specific category; PD = pharmacodynamics; sCTX = serum C-telopeptide of type 1 collagen.

ANCOVA was performed on log-transformed data of $AUEC_{0\text{-}6\ M}$ of sCTX with treatment group as fixed effect, and baseline sCTX value as a covariate. Two participants in the PD set were excluded from the ANCOVA due to negative AUEC values.

Equivalence was established if 90% CIs of geometric mean ratio lied between 0.8 and 1.25.

Source: CSR ALK22/ENZ215-DEN2 Table 11-4

Table 15. Analysis of covariance (ANCOVA) of AUEC of percentage change in sCTX from baseline to month 6 with exclusion of patients meeting EMA suggested exclusion criteria (PD Set)*

Treatment Group	n	Geometric Mean	95% CI	Ratio of Geometric Means	90% CI of geometric mean ratio	95% CI of geometric mean ratio
ENZ215 (N = 234)	196	346616.240	338467.3570, 354961.3143	1.009	0.9812, 1.0386	0.9758, 1.0443
Prolia (N = 237)	190	343365.269	335167.1715, 351763.8894			

AUEC = Area Under the Effect Curve; N = The total number of patients in PD Set after exclusion of subjects meeting EMA suggested exclusion criteria; n = Number of patients with non-missing data within the specific Category; CI= Confidence Interval.

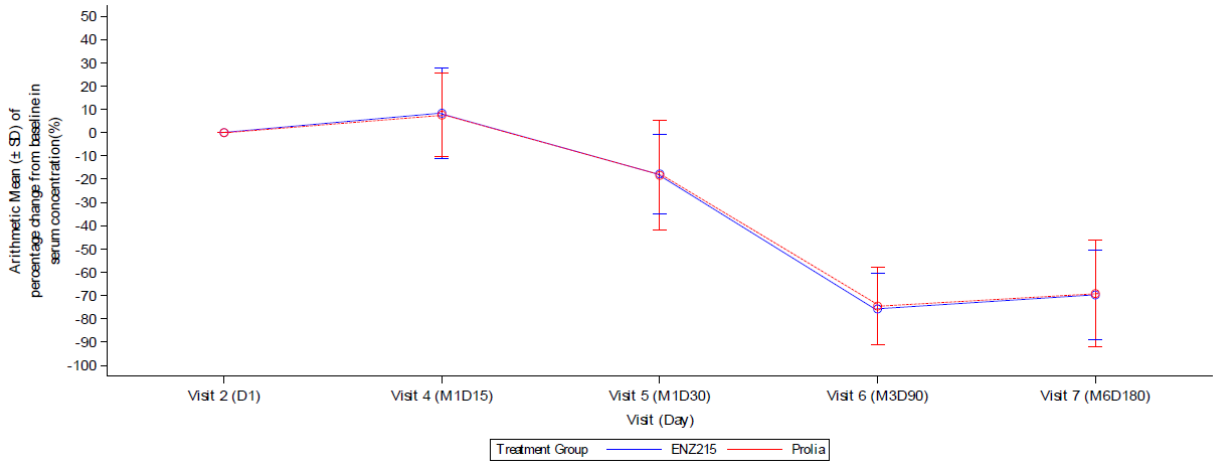
*: Patients with "PD sample collection outside the pre-defined time window (7.30 AM to 10 AM), patients "who used disallowed medications according to Protocol sect. 6.11 (at any timepoint)" and patients for "whom non-IP administration compliance (i.e., calcium and Vitamin D supplements) was <90%" are excluded from the analysis set.

ANCOVA is performed on log-transformed data of AUEC0-6m of sCTX with treatment group as fixed effect, and baseline sCTX value as a covariate after multiple imputation of missing AUEC data.

Source: Responses #2, Appendix 6

sP1NP (secondary endpoint):

Figure 6. Arithmetic Mean (± SD) for percentage change from baseline in sP1NP serum concentration of ENZ215 and Prolia groups- nominal time data -(linear scale) (PD set)



PD = Pharmacodynamic; SD = Standard Deviation; sP1NP = Procollagen Type 1 N-terminal Propeptide.

Source: Listing 16.2.8.5 and Table 14.2.2.4.

Table 16. Percentage Change in sP1NP Concentrations from Baseline Over Time by Treatment Group (PD Set)

		ENZ215 (N = 234)				Prolia (N = 237)			
Visit	**Statistics**	**Baseline**	**Post-baseline**	**Change from Baseline**	**Percentage Change from Baseline (%CfB)**	**Baseline**	**Post-baseline**	**Change from Baseline**	**Percentage Change from Baseline (%CfB)**
Baseline (D1)	n	234				236			
	Mean	63.25				63.59			
	SD	24.765				24.190			
	Minimum	16.9				10.9			
	Median	60.50				60.90			
	Maximum	161.0				166.0			
Visit 4 (M1D15)	n	234	234	234	234	235	235	235	235
	Mean	63.25	66.82	3.58	8.3369	63.60	67.21	3.61	7.5198
	SD	24.76	24.093	12.789	19.5170	24.241	24.938	10.960	17.9673
	Minimum	16.9	19.5	-70.4	-56.455	10.9	10.8	-39.6	-54.412
	Median	60.50	64.60	4.65	8.5755	60.60	63.40	3.60	5.3570
	Maximum	161.0	158.0	34.2	85.930	166.0	172.0	47.5	98.344
Visit 5 (M1D30)	n	235	235	235	235	236	236	236	236
	Mean	63.25	50.26	-12.98	-17.7485	63.59	50.00	-13.59	-18.3767
	SD	24.765	18.143	13.268	17.1910	24.190	19.204	12.940	23.4844
	Minimum	16.9	14.9	-80.3	-58.636	10.9	12.7	-50.3	-69.301
	Median	60.50	48.60	-10.90	-18.5170	60.90	46.50	-12.25	-20.4700
	Maximum	161.0	130.0	16.0	43.363	166.0	164.0	44.9	229.082
Visit 6 (M3D90)	n	233	233	233	233	236	236	236	236
	Mean	63.25	13.91	-49.34	-75.7738	63.59	14.39	-49.19	-74.5419
	SD	24.819	7.114	23.198	15.4099	24.190	6.200	22.689	16.6630
	Minimum	16.9	0.0	-135.1	-100.000	10.9	0.0	-140.1	-100.000
	Median	60.40	14.50	-47.10	-77.1380	60.90	14.35	-45.65	-76.8230
	Maximum	161.0	57.6	-3.3	-14.602	166.0	40.6	7.1	47.651
Visit 7 (M6D180)	n	233	233	233	233	234	234	234	234
	Mean	63.33	16.82	-46.51	-69.7567	63.65	17.16	-46.49	-69.1690
	SD	24.789	7.972	24.689	19.4086	24.268	8.616	23.525	22.8816
	Minimum	16.9	0.0	-141.0	-100.000	10.9	0.0	-140.1	-100.000
	Median	60.60	16.00	-43.40	-72.4680	60.90	16.05	-43.65	-72.5775
	Maximum	161.0	51.2	12.2	40.803	166.0	50.7	10.2	93.578

Abbreviations: %CfB = percentage change from baseline; D = day; M = month; PD = pharmacodynamics; N = total number of participants in PD Set; n = number of participants with non-missing data within the specific category; SD = standard deviation; sP1NP = procollagen type 1 N-terminal propeptide.
%CfB in serum P1NP was computed as, %CfB = (P1NP timepoint- P1NPbaseline/P1NP baseline) *100.
Source: CSR ALK22/ENZ215-DEN2 Table 11-5

2.5.3. Discussion on clinical pharmacology

The clinical development program of ENZ215 included one pivotal Phase 1 PK/PD study (ALK22/ENZ215-DEN1) to demonstrate bioequivalence using PK parameters, and one pivotal Phase 3 efficacy/safety/PK/PD/ADA study

(ALK22/ENZ215-DEN2), in which efficacy and a PD marker were co-primary endpoints. In both clinical studies, ENZ215 60 mg/ml PFS presentation was used as the test product which corresponds to EU- and US-Prolia 60 mg/ml PFS. No drug interaction studies, or studies in special populations, such as hepatic or renal impairment, were performed. This is acceptable for biosimilars. During the development of ENZ215, the Applicant sought Scientific Advice from the EMA Scientific Advice Working Party (SAWP) twice. Overall, the clinical pharmacology data package covers relevant studies for an application of a biosimilar medicinal product.

Bioanalytical Methods

The Applicant has adopted a direct enzyme-linked immunosorbent assay (ELISA) to quantitate ENZ215 and EU/US-Prolia (denosumab). The presented assay for determination of denosumab in human serum of healthy volunteers and patients with osteoporosis was well described and established. There are individual PK curves in the Phase 1 study which are of unusual shape and seem biologically implausible (please refer to the discussion of PK fluctuations below).

The methods for the determination of the PD parameters CTX1 (C-terminal telopeptide) and P1NP (Procollagen-type 1 N-terminal-propeptide) in human serum are based on an electrochemiluminescence immunoassay (ECLIA). The assays were well described and established, and the presented methods appear to be suitable for the determination of CTX1 and P1NP in human serum.

The Applicant has adopted an ECLIA bridging assay to screen, confirm and quantify denosumab specific antibodies in human serum matrix. The adopted three-tiered approach for determination of ADAs was well described and developed. The presented method can be considered as valid.

Further, the Applicant presented an electrochemiluminescence (ECL) assay for detection of anti-denosumab neutralizing antibodies in human serum. The presented assay was well described and validated.

Pharmacokinetics

Phase 1 Study ALK22/ENZ215-DEN1

Study design: The overall study design of ALK22/ENZ215-DEN1 (randomized, double-blind, three-arm, parallel-group, single-dose) was discussed and endorsed during scientific advice procedures.

Study population: The Phase 1 study ALK22/ENZ215-DEN1 was conducted in healthy adult male subjects. The Applicant raised the lower age limit for inclusion to 28-55 years of age (instead of 25-55 years) which is acceptable since all recruited subjects should be skeletally mature. The range for the BMI was also slightly adapted, which is acceptable.

Since smokers (< 10 cigarettes or equivalent per day within 90 days prior to screening and during the study) were allowed in the study, solid documentation of the actual tobacco consumption was recommended by the CHMP during a scientific advice procedure. Nevertheless, smoking was not sufficiently documented by study participants. Since denosumab is not metabolized by CYP enzymes, no considerable impact on PK is expected. Contrarily, a potential effect on bone mass might have decreased homogeneity of the study population and PD bone marker measurements. Concerning the documentation of tobacco consumption, a major protocol deviation was documented in multiple subjects (i.e., cotinine testing was not consistently performed; please also refer to section "Study conduct" below). Although no solid documentation of tobacco use was provided by the Applicant, cotinine levels of > 500ng/ml would have only detected heavy smokers and would have not been regarded suitable/sensitive to check for the compliance to tobacco limits before and during the study. Therefore, the lack of tobacco consumption documentation is critically noted, nevertheless, not further pursued for the DEN1 study.

Overall, the study population is deemed representative, sufficiently homogeneous and sensitive to demonstrate PK biosimilarity.

Study treatment: During a scientific advice procedure (15 Oct 2020), the use of a 60mg dose was considered acceptable given partial AUCs are investigated.

Administering IPs at a single site of injection at the upper thigh was considered acceptable during scientific advice. It is critically noted that, apparently, no calcium and vitamin D supplements were provided for all participants, although the Prolia SmPC states that "Patients must be adequately supplemented with calcium and vitamin D". Not providing supplements might have increased heterogeneity in bone metabolism. Nevertheless, providing supplements was not a requirement for the PK study. Since the study is already completed and serum calcium was monitored, the issue is not further pursued.

Study objectives and endpoints: The Applicant followed the CHMP's recommendation from scientific advice procedures of using AUCinf and Cmax as co-primary endpoints for this single dose study with subcutaneous (s.c.) administration. Additionally, AUC0-t was considered as third co-primary endpoint by the Applicant but is interpreted as secondary endpoint in this assessment. Overall, study objectives and endpoints are acceptable.

During a scientific advice procedure, the use of a 60mg dose was considered acceptable. In the case of using that dose, the Applicant was advised to also analyse partial AUCs reflecting the different elimination pathways (non-target-mediated vs target-mediated) or to employ PK modelling to support the assessment of PK similarity between the studied products (PK Q&A, EMA/CHMP/SAWP/338801/2019). The Applicant provided AUC(0-28d) which is considered useful to characterize the *absorption* phase. Upon request, the Applicant provided post-hoc analyses of partial AUCs for the interval where it is expected that target-mediated clearance starts to predominate (see below).

Sampling time points for PK and PD sampling were endorsed during scientific advice. PK sampling time points seem to adequately cover the time around Cmax, i.e., 10 days (range 2-28 days) and both the early (day 28 until day 180) and late (until day 270) elimination phases. Furthermore, the time points for PD (CTX) assessment were considered acceptable. it was agreed that the follow-up over 9 months adequately covers the PD profile (CTX) of denosumab.

Sample size: The sample size calculation was performed individually for the parameters AUC0-t, AUC0-inf and Cmax assuming a true ratio of 0.95 and a common coefficient of variation of 33.5%. As there were three co-primary parameters, it would have been more prudent to target the conjunctive power instead of the individual power, but considerations on the sample size calculation are of little relevance after conduct of the study.

Randomization: Subjects were randomly assigned to one of the three study arms (1:1:1) to receive either ENZ215, EU-sourced Prolia or US-sourced Prolia. The randomization scheme did not take into account any stratification factors. Generally, the process of randomization was adequately described and is considered acceptable.

Blinding: Study ALK22/ENZ215-DEN1 had a double-blind design. The randomization was made available only to the clinic pharmacy staff preparing the drug who were not involved in any other aspect of the study including administration of the drug. No unblinding occurred during study conduct. The process of blinding is considered adequate.

Statistical methods: Applying an analysis of variance to the log-transformed primary endpoints Cmax and AUC0-inf and the secondary endpoint AUC0-t with treatment group as factor is suitable to determine

equivalence in terms of PK endpoints. Equivalence was to be determined if 90% CI for the ratio of geometric LSMeans of ENZ215 to EU sourced Prolia was within the equivalence range of 0.80 to 1.25, which is in line with the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **).

Study conduct: Three study centers in 3 countries (Bulgaria, Poland, and United Kingdom) consented at least 1 subject. Adding further study centers after the start of recruitment due to challenges in recruiting subjects is considered acceptable. In addition to adding study centers, three non-administrative protocol amendments have been made after the start of subject recruitment. In general, the changes in the three protocol amendments are not considered to have a drastic impact on the study results.

In total, 193 "minor" and 51 "major" deviations occurred in the DEN1 study. Of 207 randomized subjects, 116 subjects had "minor" deviations and 28 subjects out of them had "major" deviations (10 subjects in ENZ215 group and 9 subjects each, in EU-sourced Prolia and US-sourced Prolia groups). All the "major" deviations were procedure related which included informed consent form (ICF) related, missing visits, additional cotinine test was not performed, and COVID-19 vaccination timing. Those "major" protocol deviations are acknowledged. Upon request, the Applicant provided a summary table and listing of protocol deviations from the DEN1 study. Three main factors were stated which contributed to the high rate of protocol deviations, i.e., the tight time windows for assessments; multiple and frequently overlapping assessment requirements for PK, PD, immunogenicity, safety etc; and the 16-month study duration involving healthy volunteers which led to logistical and compliance challenges. The Applicant argued that HVs, unlike patients with medical conditions, may have less motivation to remain committed to the study for a prolonged timeframe.

Subject disposition, demographics and baseline characteristics: Overall, subject disposition is deemed acceptable, and demographic and baseline characteristics are considered similar between groups.

Study outcomes: The geometric LSMean ratios (90% CI) for Cmax, AUC0-inf, and AUC0-t comparing ENZ215 to EU-sourced Prolia were 98.97 [91.52, 107.03], 97.98 [89.82, 106.89], and 97.83 [89.60, 106.81], respectively. Thus, the 90% CIs for all primary PK parameters were entirely contained within the prespecified margins of 80% and 125%. Overall, the primary PK endpoints of Study ALK22/ENZ215-DEN1 were met, supporting biosimilarity. Furthermore, no notable treatment differences were observed in the secondary PK parameters ($pAUC_{0\text{-}D28}$, T_{max}, $T_{1/2}$, and CL/F).

Although no sensitivity analysis for the primary PK endpoints (Cmax and AUC_0–inf; excluding all samples affected by 'minor' protocol deviations related to PK laboratory assessments, study procedures, or visit schedules) is available, the PK results from study DEN1 are considered sufficiently robust to support a conclusion of biosimilarity.

This conclusion is supported by several factors:

- The reassessment of the amended protocol deviation listing did not identify any deviations of critical concern.
- A provided comprehensive listing of individual minor protocol violations allowed traceability and clarity of individual deviations, allowing for better contextual understanding.
- Despite the overall high number of protocol deviations, the predefined primary PK endpoints showed clear and consistent results.
- A systematic evaluation of individual PK profiles was conducted to assess fluctuations.

The Applicant provided mean serum concentration vs nominal time curves. Overall, profiles were comparable between all groups. However, from ~2832 h (Day 119), mean concentrations of EU- and US-Prolia decreased slightly faster compared to ENZ215. As the PK profile of denosumab is known to show a more rapid terminal elimination due to target-mediated drug disposition (TMDD) at lower concentrations, the observed difference may suggest differences during the target-mediated part of denosumab elimination. Upon request, the Applicant provided post-hoc analyses of partial AUCs for the intervals between weeks 0-17 and weeks 17-38 (i.e., the interval where it is expected that target-mediated clearance starts to predominate). While ENZ215 and EU-sourced Prolia showed similar exposure for the $pAUC_{weeks0-17}$, the LSM ratio (90%CI) between $pAUCs_{weeks17-38}$ was 118.73% (40.75% to 345.93%), indicating high inter-subject variability (CV: 86,920.5%) and reduced precision in estimates. According to the Applicant, the higher rate of between-subject variability resulted in higher incidence of below the limit of quantification (BLQ) during this time frame. Due to the high inter-subject variability, no conclusions are drawn based on the $pAUC_{weeks17-38.}$

PK fluctuations: In some of the subjects' individual serum concentration profiles, a sudden fluctuation in concentration was observed around time point 120h. Except for participant ENZ215/02-042, all of those spikes apparently returned to the previous level at the next evaluation time point. Such PK spikes occur at both, test and reference product, e.g., in at least three ENZ215 participants and two Prolia participants with clearly visible fluctuations. In addition to these plots of those five subjects (shown in the 2.5.2.1.1. "Outcomes" section), there are further subjects which have PK curves showing a less pronounced fluctuation between 72 and 480 hours after dosing. These patterns raised questions regarding the origin of these observations as well as concerns regarding the validity of the conclusions made from standard equivalence assessments on such PK-concentration read-outs. The proximity of these spikes to Tmax questioned the appropriate coverage of Tmax and Cmax, and potentially also might have impacted AUC computation at the individual subject level.

Upon request, the Applicant conducted a root cause analysis (RCA), examining potential contributing factors across three domains: Clinical pharmacology (biological variability, subject history, subject compliance, etc.), clinical operations (sample handling and logistics, sampling technique, etc.), and bioanalytical data (testing procedures, data review, etc.).

The RCA did not reveal any significant abnormalities in clinical pharmacology or clinical operations. However, an issue was identified in the bioanalytical data: three samples from a single subject were impacted, with one replicate falling outside the analytical range. These aberrant results were not flagged for re-assay by the Bioanalytical Project Investigator (BPI), and the routine quality control (QC) review also failed to detect the anomaly. A subsequent QA audit of the raw data and associated report found no evidence of systematic error. Following another request, an additional sample from another subject was found to be non-reportable due to high CV. It is critically noted that these samples had not been flagged earlier, but nevertheless this does not impact the overall PK analysis and is therefore acceptable.

In general, administration related errors were ruled out by the Applicant since no deviations were recorded during dosing in the current study with subcutaneous administration using pre-filled syringes in the DEN1 study according to the Applicant's investigations. Assay performance was also reviewed critically. The bioanalytical method was fully validated and met the overall requirements for all tested validation assessments and was thus fit for purpose. The acceptance criteria were met for all the tested parameters. Additionally, all the sample analysis runs met the assay acceptance criteria confirming the validity of the results. Moreover, values obtained for the affected samples which were part of ISR were similar to the original results. This makes assay variation or interference less likely to be responsible for the observed pattern in serum concentration time profile. Furthermore, the data points before and after dip/peak were in

agreement with expected disposition pattern (steady increase towards the maximum concentration before Cmax or a decrease following the maximum concentration).

In addition to the investigations, the Applicant conducted a literature review to understand the basis of observed fluctuations. Reijers et al. (2016), investigated three registered (adalimumab, bevacizumab, and trastuzumab) and three unregistered immunoglobulin G1 mAbs. Interestingly, a high sample density was associated with an increased likelihood of detecting additional peaks. The authors concluded that there was no difference in occurrence between the high- and low-concentration ranges. Also, assay variability or interference was considered unlikely to explain fluctuations. Of note, depicted PK profiles in the study by Reijers et al. had different shapes and less clear fluctuations, making a direct comparison with the denosumab IP questionable. In addition to assay-related considerations, multiple physiological mechanisms could hypothetically be a source for mAbs variability (Reijers et al., 2017, 2018; Ingvar et al., 1990; Aldrich et al., 2017; Reitsma et al., 2007; Sutjandra et al., 2011; Vogg et al., 2024; Lee et al., 2023; Waldmann 1969), e.g.: retention of mAbs in lymph nodes related to sleep/wake phase, redistribution of mAbs via the capillary bed of lymph nodes, fluctuated expression of neonatal Fc receptor for IgG (FcRn) in organs, adsorption to the luminal surface of endothelial cells, or fluctuating plasma volume. Summarizing, it is acknowledged that PK fluctuations were also observed with other mAbs in the literature due to multiple potential reasons, including sampling, assay and physiological considerations.

To evaluate the impact of these fluctuations, two different sensitivity analyses (sensitivity analysis #1 excluding subjects with fluctuations & sensitivity analysis #2 excluding only sample identified as fluctuations) were performed. A fluctuation was flagged if the concentration at a given timepoint deviates by more than ±30% relative to both its preceding and succeeding values. The analysis identified 10 samples with fluctuations. In both analyses, the 90% CIs for all parameters remained fully within the predefined bioequivalence acceptance range of 80.00% to 125.00%. These findings confirm the robustness of the primary PK results from the DEN1 study and demonstrate that the observed PK fluctuations - regardless of whether subjects or only individual time points for these were excluded - did not impact the conclusion of bioequivalence between ENZ215 and EU-Prolia.

Summarizing, no single, clear root cause for the PK fluctuations could be identified. The Applicant's conclusion is considered acceptable: based on the sensitivity analyses and evaluations from clinical pharmacology, clinical operations, and bioanalysis, the observed fluctuations do not impact the bioequivalence conclusion between the test and reference products.

Phase 3 Study ALK22/ENZ215-DEN2

In addition to the dedicated PK study ALK22/ENZ215-DEN1, the Applicant also provided PK data from the Phase 3 Efficacy study ALK22/ENZ215-DEN2. For the DEN2 study, PK parameters were only analysed descriptively, which is acceptable, given the supportive nature of the data.

The mean serum concentration-time profiles were comparable between ENZ215 and EU-Prolia group at baseline (Day 1), Day 8, Day 15, Month 1, Months 3, and Month 6 (prior to second dose), and Ctrough at Month 12 (after the second dose). PK data from the Phase 3 study ALK22/ENZ215-DEN2 are only regarded supportive, especially due to the sparse sampling time points around Tmax. In fact, median Tmax was almost exactly five days in healthy subjects the DEN1 study, while it appeared to be about 14 days in osteoporosis patients in the DEN2 study. According to the Prolia SmPC, maximum serum denosumab concentrations occurred after 10 days (range 2-28 days) for a 60 mg subcutaneous dose. Therefore, it is agreed with the Applicant that study populations (DEN1: healthy, adult males, 25-55 yoa; DEN2: post-menopausal women with osteoporosis, 55-85 yoa) and designs (single vs. multiple doses), study endpoints and assessments do

not allow a direct comparison of results across the 2 studies (Study ALK22/ENZ215-DEN1 and Study ALK22/ENZ215-DEN2).

Cmax, AUC0-1M, AUC0-3M and AUC0-6M were roughly comparable between treatment groups, although geometric mean values were by about 2-13% higher in the ENZ215 group compared to the Prolia group. Since there was no sampling time point between Month 6 and 12, AUC_{6M-12M} is not regarded meaningful. In summary, denosumab PK parameters are regarded comparable between ENZ215 and Prolia groups within the DEN2 study. Nevertheless, sparse sampling and below limit of quantification values make it difficult to conclude much from the PK analysis in the phase 3 study. Reassuringly, there is no indication that there were significant differences between the groups in PK values. Furthermore, comparability between studies seems limited, likely due to differences in study populations, study design, and, especially, due to the sparse PK sampling in the Phase 3 study.

Additionally, median denosumab $C_{trough\ 6M}$ and $C_{trough\ 12M}$ were below the lower limit of quantification (LLOQ) in both treatment groups in the DEN2 study. Since more than half of patients had values below the LLOQ, and since the coefficient of variation was very high, no further conclusions can be drawn from the analysis.

Overall, the PK characterization in the Phase 3 DEN2 study is considered acceptable. The PK profiles of osteoporosis patients support PK similarity of the test and reference product.

Pharmacodynamics

The pharmacodynamics of ENZ215 and Prolia have been investigated in two studies (ALK22/ENZ215-DEN1 (healthy adult men); ALK22/ENZ215-DEN2 (post-menopausal women with osteoporosis)). Apart from these studies, no other clinical pharmacology studies (i.e., drug interaction studies, or studies in special populations such as hepatic or renal impairment) were performed.

Mechanism of action: ENZ215 was developed as a biosimilar product to Prolia. The mechanism of action is identical to the reference products. Based on the same mechanism of action, extrapolation to all indications is justified, provided that similarity is shown regarding quality and extended functional characterization and that clinical data show comparability in terms of PK, PD, efficacy and safety.

Phase 1 Study ALK22/ENZ215-DEN1

CTX-1: The AUEC was to be calculated as the area under the effect curve from baseline until CTX-1 values returned to baseline for the first time, i.e. the AUEC could not take negative values. This analysis did not take into account the rebound area, i.e. the area that is below 0 and above the %CfB in sCTX curve. However, the results reveal that only very few subjects had sCTX values above the baseline value, i.e. negative %CfB values and thus non-zero rebound area. Thus, it is agreed that rebound area can be ignored for the PD assessment. Of note, in the phase 3 study, the AUEC was defined as the net area, i.e. could take negative values. An analysis of covariance was to be performed on the log-transformed AUEC, including treatment as factor and baseline CTX-1 value as covariate.

Similarly, as for the PK parameters, there is some discrepancy in the description of which subjects were to be included in the pharmacodynamic analysis. On the one hand, the pharmacodynamic analysis set might have included subjects with only one post-dose measurement. On the other hand, it was stated that the AUEC would not be calculated in case of three consecutive missing values during the late phase. This issue might be related to an inconsistency between the number of patients included in the descriptive and the inferential analysis. According to Table 19, the descriptive analysis of the area under the effect curve AUEC0-270 for serum CTX-1 percent inhibition included only 182 subjects (88.3%) of the 206 subjects the PD population, while the ANCOVA model of AUEC0-270 was reported to include the full PD population. Usually, it is expected

that the patients included in the model analysis are a subset of the patients included in the descriptive analysis. However, as the PD data from the phase 1 study are not considered pivotal, this issue is not further pursued.

For the Area under the effect curve (AUEC) from time 0 to Day 270 for serum CTX-1 percent inhibition ENZ215 demonstrated a geometric mean ratio of 0.98 with a 95% CI of [0.92, 1.03] when compared to EU Prolia in the ALK22/ENZ215-DEN1 study. The 95% CI of the GMR for AUEC is considered sufficiently close around unity to conclude on PD similarity. Similarly, when comparing ENZ215 to US Prolia, ENZ215 showed a geometric mean ratio of 0.99 with a 95% CI of [0.93, 1.05].

Phase 3 Study ALK22/ENZ215-DEN2

CTX-1 (co-primary endpoint): The area under the effect curve of percentage change from baseline in sCTX from baseline to month 6 was one of the two co-primary parameters in the DEN2 study. It is understood that the AUEC was calculated as the net area under the effect curve from baseline to month 6.

PD parameters were to be analyzed on the PD set, which was defined to include all randomized patients who received at least one dose of study intervention, whose sCTX values were available in order to calculate PD parameter AUEC values for primary analysis and had no major protocol deviations which affected sCTX or sP1NP measurement.

An ANCOVA model on the log-transformed AUEC values including treatment group and baseline sCTX as independent variables was used to compare the treatment arms. Pharmacodynamic equivalence was to be concluded if the 90% CI of the treatment ratio was contained within the acceptance limits of 80% to 125%. 95% CIs of geometric mean ratio between the treatment groups also were to be provided. The proposed acceptance range of 80-125% for the PD endpoint AUIC0-6months is based on margins used for conventional bioequivalence analyses without further justification. The acceptance range of 80–125% is not appropriate per se, but as the provided results are considered clear enough to support equivalence, this issue is not further pursued. Moreover, the 95% CI (instead of the 90% CI) is considered the CI relevant for assessment of pharmacodynamic equivalence.

The PD set included 234 patients (92.5% of ITT) in the ENZ215 arm and 237 patients (94.4% of ITT) in the Prolia arm. Upon request, the Applicant clarified that patients were excluded in 23 cases since the sCTX AUEC could not be calculated due to missing PD sampling at one or more critical time points, i.e., Days 1, 15, 30 or 180 (17+6 cases). Additional exclusions were due to missed collection of Day 30 PD samples (2 cases), use of prohibited medications (6 cases), and patients meeting exclusion criteria (2 cases).

The Applicant provided the mean percent change from baseline in sCTX concentration for the PD set. CTX baseline levels were similar between the treatment groups. Overall, the profiles appear comparable between ENZ215 and the EU-Prolia group. At Visit 5 (D30), the standard deviation of values in the Prolia group is at least about three times higher than the standard deviation at any other visit in the ENZ215 group or the Prolia group.

Compared to other patients in the Prolia treatment arm (mean baseline sCTX: 0.4904 ng/mL), one patient exhibited a markedly lower baseline sCTX value (0.051 ng/mL). By Visit 5 (Day 30), the mean sCTX level in the Prolia group had decreased to 0.0443 ng/mL, whereas patient 60301005 showed a paradoxically elevated sCTX concentration of 0.819 ng/mL. Upon request, the Applicant investigated the extreme %CfB in sCTX observed for this patient at Day 30 in study ALK22/ENZ215-DEN2, which was reported as -1505.9%. Despite reviewing bioassay performance, sampling procedures, potential intercurrent events (none were reported for this patient), medical history, prior antiresorptive treatments, protocol deviations, and TEAEs, no definitive root cause could be identified. It is not agreed with the Applicant's conclusion that the patient's ongoing

treatment with 100 mg/day oral aceclofenac for osteoarthritis (since September 2020) and/or the increase in elemental calcium intake from 0.4 g to 1 g daily on Day 15 could *not* have contributed to the aberrant sCTX value. However, in the absence of documented osteoarthritis exacerbation or changes in medication with an influence on bone resorption (other than calcium), no clear explanation is apparent. All anomalous sCTX values for patient 60301005 may therefore be considered outliers.

The equivalence of ENZ215 compared to Prolia was demonstrated for the primary PD endpoint of AUEC of % change in sCTX from baseline to Month 6 with a ratio of geometric mean of 1.004 and a 95%CI of [0.97485, 1.0349]. The 95%CI is considered sufficiently close around unity to conclude on PD similarity. An analysis on the ITT set using multiple imputation as requested during the assessment gave very similar results.

Upon request, using a stricter definition of the PD set (see Table 14.2.2.3b) also gave similar results to the primary analysis. This supports the robustness of the PD results and indicates that the protocol deviations observed in the DEN2 study did not compromise the conclusion of biosimilarity.

According to the study protocol, no PD/efficacy assessment was planned for subjects in the OLE until EOS2 at Month 18. Thus, no data from the open-label switch over period are available, except for immunogenicity/safety data. Although the switch data would have provided relevant information for the clinical practice, a 6-month switch over period is not a formal CHMP regulatory requirement. Therefore, it was agreed during scientific advice that a follow-up over 9 months adequately covers the PD profile (CTX) of denosumab.

sP1NP (secondary endpoint): The Applicant provided the summary statistics of P1NP serum concentration for each timepoint for the PD set. The baseline levels were similar between the treatment groups. The results for all other timepoints (Month 0.5, 1, 3, 6) were also similar for each treatment group. Thus, the results support the PD similarity of the test and reference product. No values were provided for the end of the double-blind treatment period (Month 12), which is accepted.

2.5.4. Conclusions on clinical pharmacology

For PK and PD assessment of biosimilarity the applicant widely followed scientific advice and the relevant guideline for biosimilars regarding study design and statistical analysis.

PK: In Phase 1 PK/PD study ALK22/ENZ215-DEN1 (pivotal study for PK), the geometric LSMean ratios (90% CI) of the GMRs for Cmax, AUC0-inf, and AUC0-t (FDA requirement) were 98.97 [91.52, 107.03], 97.98 [89.82, 106.89], and 97.83 [89.60, 106.81], respectively. Thus, primary PK parameters were within the pre-defined equivalence range of 0.80 to 1.25, suggesting PK equivalence. Important limitations of the study were the high rate of protocol deviations (56% and 13.5% subjects had minor and major deviations, respectively), and PK fluctuations in some of the subjects' individual serum concentration profiles. In the Phase 3 Efficacy study ALK22/ENZ215-DEN2, PK sampling was only sparse. Nevertheless, the PK profiles from the osteoporosis patients were similar between the ENZ215 and EU-Prolia group and support PK similarity of the test and reference product.

PD: In study ALK22/ENZ215-DEN1, the point estimate of the geometric mean ratio (ENZ215/EU-Prolia) for "AUEC 0-9M of percent change from baseline in serum CTX concentration" was 0.98 with the corresponding 95% CI being (0.92, 1.03) (secondary endpoint). In study ALK22/ENZ215-DEN2 (pivotal study for PD), the point estimate of the geometric mean ratio (ENZ215/EU-Prolia) for "AUEC 0-6M of percent change from baseline in serum CTX concentration" was 1.004 with the corresponding 90%CI of (0.9795, 1.0299), and 95% CI of (0.9748, 1.0349) (co-primary endpoint). The 95%-CIs of both studies are sufficiently close around

unity to conclude on PD similarity. In addition to sCTX, the levels of P1NP were comparable between the treatment groups at each timepoint, supporting PD similarity of test and reference product.

2.5.5. Clinical efficacy

During the clinical development program of ENZ215 as a proposed denosumab biosimilar of Prolia, one comparative clinical efficacy and safety trial (Study ALK22/ENZ215-DEN2) was performed in postmenopausal women with osteoporosis, an approved indication for the reference product (RMP) Prolia, to establish similar efficacy, safety, immunogenicity and pharmacodynamics (PD) between ENZ215 and the reference product. An overview of the study is provided in the table below.

Table 17: Overview of Study ALK22/ENZ215-DEN2

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
ALK22/ENZ215-DEN2	Completed First enrollment: 04 Jul 2022 Last Participant Completed: 18 Jul 2024 CSR: 28 Oct 2024 Nr. of participants enrolled: 504	Phase 3 Multicenter, Double-Blind, Randomized, Parallel-Group, Active-Controlled trial	Test Product: Denosumab 60 mg/mL in single-use prefilled syringe Reference Product: Prolia 60 mg/mL in single-use prefilled syringe Route of administration: Subcutaneous	Postmenopausal women with osteoporosis aged ≥55 to ≤85 years, globally except in Spain (where participants had to be ≥ 75 and ≤ 85 years with LS T-score ≤ -2.5 or b. LS T-score ≤ -2.5 and a prior fragility fracture (except for hip fracture), including non-exclusionary vertebral fractures c. contraindication for bisphosphonates or who do not tolerate the oral route.)

2.5.5.1. Dose response study(ies)

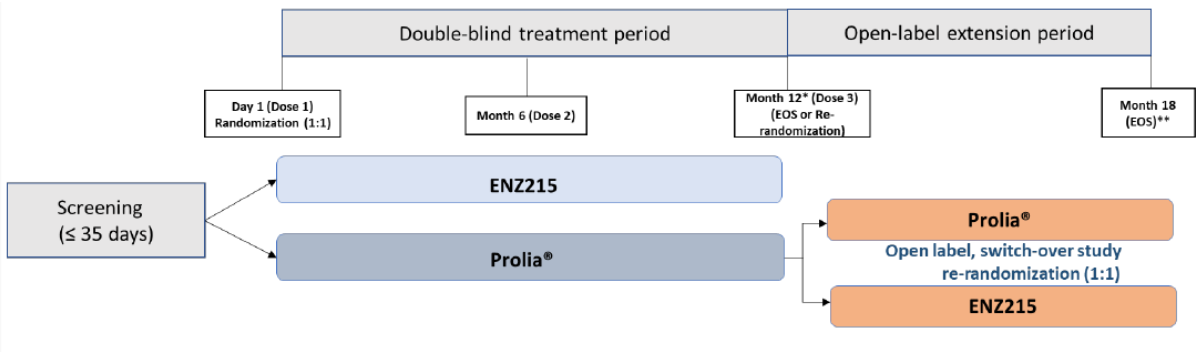
No dose-response studies were performed. Nevertheless, dose-response studies are not a requirement in the biosimilarity setting.

2.5.5.2. Main study(ies)

ALK22/ENZ215-DEN2: A Phase 3, Randomised, Double-blind, Parallelgroup, Active-controlled Study to Compare the Efficacy, Safety, Pharmacodynamics, Pharmacokinetics and Immunogenicity of Enzene Denosumab (ENZ215) and Prolia in Postmenopausal Women with Osteoporosis

The study was conducted at 44 centres that enrolled participants across the European Union (Investigators and Other Important Participants: Bulgaria [4 sites], Czech Republic [8 sites], Denmark [1 site], Lithuania [8 sites], Poland [23 sites], Serbia [6 sites], Spain [2 sites]). The study was divided into three periods: Screening period: up to 35 days; Double-blind treatment period of 12 months; and Open-label, switch-over period of six months.

Figure 7. Study schema



Abbreviation: EOS = end of study.
*Month 12 – EOS for participants randomised to ENZ215.
**Month 18 – EOS for subset of 120 participants re-randomised to receive Prolia or ENZ215.

Double-blind treatment period: All eligible participants were randomised in the double-blind treatment period in a 1:1 ratio to receive either ENZ215 or Prolia (60 mg) SC on Day 1 and Month 6. The participant allocation was stratified by age (≥ 55 to < 70 years and ≥ 70 to ≤ 85 years) and based on prior use of bisphosphonate.

A PK sub-study was conducted in a subset of 120 participants. For participants in the PK sub-study, PK samples were collected at the timepoints described in section 2.5.2.1.2.

Open-label extension period: A subset of 120 participants randomised to Prolia arm and who completed 12 months of the double-blind treatment period without any significant safety concerns per the Investigator's discretion were offered to enrol in the open-label, switch-over extension period. The purpose of this open-label extension period of the study was to assess the impact on immunogenicity and safety of switching participants from Prolia to ENZ215. After re-consenting for the open-label, switch-over study, the participants were re-randomised, without any stratification, in a 1:1 ratio to receive either ENZ215 or Prolia (60 mg) SC at Month 12. These participants completed the study at Month 18.

Methods

- **Study Participants**

This study enrolled postmenopausal women with osteoporosis aged ≥ 55 and ≤ 85 years across the European Union.

Inclusion Criteria

1. Willing to provide voluntary written informed consent and able to comply with the protocol requirements
2. Postmenopausal women aged ≥ 55 and ≤ 85 years globally, except for Spain. In Spain specifically refer to the below criteria:
    a. Postmenopausal women aged ≥ 75 and ≤ 85 years with LS T-score ≤ -2.5 or

b. Postmenopausal women aged ≥ 65 and < 75 years with LS T-score is ≤ -2.5 and a prior fragility fracture (except for hip fracture), including non-exclusionary vertebral fractures

c. In both cases (i.e. criteria a and b), it must also be that these are women who present a contraindication for the use of bisphosphonates or who do not tolerate the oral route.

3. Body weight ≥ 50 kg and ≤ 90 kg
4. Diagnosed with osteoporosis, with absolute BMD consistent with T-scores of ≤ -2.5 and ≥ - 4.0 at the lumbar spine (L1-L4 region) as measured by dual-energy X ray absorptiometry (DXA) at screening
5. At least 5 years of postmenopausal status confirmed by follicle-stimulating hormone (FSH) levels at screening
6. At least one hip joint and two vertebrae in L1-L4 region evaluable by DXA
7. No other clinically significant medical history, vital signs, physical examination, laboratory profiles as deemed by the Investigator or designee that would pose a risk to participant safety or interfere with the study evaluation, procedures or completion

Exclusion Criteria

1. Known hypersensitivity to denosumab or any of the excipients of the study drug
2. Known intolerance to, or malabsorption of calcium or vitamin D supplements
3. Previous exposure to Prolia or any other denosumab biosimilar
4. Previous use of oral bisphosphonates:
   a. Used for 3 or more years cumulatively
   b. If used for < 3 years, use within the past 12 months prior to screening
5. Use of intravenous bisphosphonates within the past 5 years prior to screening. If used more than 5 years prior, patients will be excluded if cumulative use was > 3 years.
6. Use of parathyroid hormone or its derivatives, hormone replacement therapy, romosozumab, selective estrogen-receptor modulators, or tibolone or calcitonin within 12 months prior to enrollment
   Note: occasional use of intravaginal estrogen treatment is not exclusionary
7. Any prior use of fluoride or strontium
8. Systemic glucocorticoids (≥ 5 mg prednisone equivalent per day or cumulative dose ≥ 50 mg) for more than 10 days within 3 months prior to enrollment (topical and inhaled corticosteroids are allowed)
9. Other bone active drugs (i.e. drugs affecting bone metabolism) including heparin, anti-epileptics (except for benzodiazepines and pregabalin), antidepressants such as SSRIs, SNRIs, antipsychotics, systemic ketoconazole, adrenocorticotrophic hormone (ACTH), lithium, protease inhibitors, gonadotropin-releasing hormone (GnRH) agonists, or anabolic steroids within the past 3 months prior to screening or requiring treatment with these agents during the study.
   Note: Please refer to section 3.3.1.1.4 "Concomitant and rescue therapies" of this report for a comprehensive list of prohibited medications

10. Known sensitivity to drug products derived from mammalian cell lines such as hormones, enzymes, cytokines, bone morphogenic proteins, clotting factors, antibodies, and fusion protein therapeutics. Patients with any known hypersensitivity to complex proteins such as monoclonal antibodies will be excluded.
11. History of one severe or more than two moderate vertebral fractures per Genant classification as determined by the central reading center
12. History of hip fracture or bilateral hip replacement
13. Total hip or femoral neck T-score <4.0
14. History and/or presence of atypical femoral fracture
15. Presence of any active healing fracture according to the Investigator's assessment
16. History of any transplant or chronic immunosuppression (including patients on immunosuppressive therapy)
17. Severe liver dysfunction (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] > 3 times upper limit of normal)
18. Positive testing for hepatitis B (hepatitis B virus surface antigen [HbsAg]) or hepatitis C (hepatitis C virus antibody [HCV Ab]) virology
19. Known history of human immunodeficiency virus (HIV) infection or positive serology for HIV at screening
20. Significantly impaired renal function (determined by glomerular filtration rate of < 45 mL/min/1.73 m2 by the Modification of Diet in Renal Disease (MDRD) formula, as calculated by the central laboratory) or receiving dialysis
21. Oral or dental conditions:
    a. Osteomyelitis or history and/or presence of osteonecrosis of the jaw (ONJ)
    b. Presence of risk factors for ONJ (e.g., periodontal disease, poorly fitting dentures, poor oral hygiene, invasive dental procedures such as tooth extractions within 6 months prior to screening)
    c. Active dental or jaw condition which requires oral surgery
    d. Planned invasive dental procedure
22. Major surgery within 8 weeks prior to screening or anticipated major surgery during the study
23. Clinically significant leukopenia, neutropenia, or anemia as determined by the Investigator or any other clinically significant medical condition or laboratory abnormality that, in the opinion of the Investigator, would pose a risk to patient safety or interfere with adherence to study procedures, study completion, or the interpretation of study results
    Note: In case of an abnormal laboratory result which in the opinion of the investigator may be an error, is borderline, or indeterminate for inclusion in the study, the investigator may consider repeating the test once in order to rule out laboratory error.
24. Patient with an active infection or history of infection as follows:

a. Any active infection for which systemic anti-infectives were used within 4 weeks prior to randomization

b. A serious infection defined as requiring hospitalization or intravenous anti-infectives within 8 weeks prior to randomization

c. Recurrent or chronic infections or other active infection that, in the opinion of the Investigator, might compromise the safety of the patient

25. Evidence of any of the following conditions per laboratory test results, medical history, electrocardiogram (ECG), DXA, or X-ray review:

a. Uncontrolled hyperthyroidism or hypothyroidism

Note: Clinical significance of abnormal TSH values in patients on stable replacement therapy due to hypothyroidism or on anti-thyroid medication should be assessed and discussed with the Medical Monitor.

b. History or current hyperparathyroidism or hypoparathyroidism (intact parathyroid hormone levels not within normal range)
Note: Mild secondary hyperparathyroidism in the context of vitamin D deficiency may be acceptable upon discussion with the Medical Monitor.

c. Vitamin D deficiency defined as 25 (OH) vitamin D level < 20 ng/mL (< 50 nmol/L)
Note: Patients can be enrolled if a repeat test (post supplementation) prior to enrollment shows corrected 25 (OH) vitamin D level ≥ 20 ng/mL (≥ 50 nmol/L).

d. Current hypocalcemia (albumin-adjusted serum calcium < 8.0 mg/dL [< 2.0 mmol/L]) or hypercalcemia (albumin-adjusted serum calcium > 10.6 mg/dL [> 2.62 mmol/L])

e. History of parathyroid surgery

f. Any bone or metabolic disease which may affect BMD or interfere with the interpretation of the findings, e.g., osteomalacia, osteogenesis imperfecta, osteopetrosis, achondroplasia, Paget's disease, rheumatoid arthritis, ankylosing spondylitis, Cushing's disease, hyperprolactinemia, or malabsorption syndrome

g. Any malignancy, including solid tumors, and hematologic malignancies (except basal cell carcinoma and squamous cell carcinomas of the skin, cervical, or breast ductal carcinoma in situ, that have been completely excised and are considered cured) within the last 5 years

h. Known or suspected history of alcoholism (including heavy drinking defined as consuming more than 3 drinks on one day or more than 7 drinks per week) or substance abuse within the past 12 months prior to the first dosing that the Investigator believes would interfere with understanding or completing the study

i. Current heavy smoking, defined as smoking 20 or more cigarettes per day

j. Participated in any other clinical study in last 30 days prior to screening

k. History and/or presence of significant cardiac disease as per Investigator's discretion, including but not restricted to:

i. History of cardiac arrhythmia or long QT syndrome or ECG abnormalities at screening indicating significant risk for safety (e.g., that required hospitalization, emergency cardioversion, or defibrillation)

ii. History and/or presence of myocardial infarction within 6 months before screening

iii. History and/or presence of New York Heart Association (NYHA) class III or IV heart failure

26. Suspected signs and symptoms of COVID-19/confirmed COVID-19 or with recent history of travel/contact (less than 2 weeks from screening) with any COVID-19 positive patient/isolation/quarantine

- **Treatments**

Table 18. Study Intervention, Dose and Mode of Administration

Intervention Label	ENZ215	Prolia	Vitamin D	Calcium
Intervention Name	ENZ215	Prolia	N/A	N/A
Type	Biologic	Biologic	Drug	Drug
Dosage Formulation	PFS	PFS	Tablet	Tablet
Unit Dose Strength(s)	60 mg/mL	60 mg/mL	400 IU daily	1 g daily
Dosage Level(s)	60 mg	60 mg	At least 400 IU	At least 1 g
Route of Administration	SC injection	SC injection	Oral	Oral
Use	Experimental	Active comparator	Supplement	Supplement
IMP or NIMP	IMP	IMP	NIMP	NIMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	Locally sourced	Locally sourced
Packaging and Labeling	Study intervention was provided in a PFS. Each PFS was labeled as required per country requirement.	Study intervention was provided in a PFS. Each PFS was labeled as required per country requirement.	Per local labelling requirement	Per local labelling requirement
Current Name	ENZ215 (denosumab)	Prolia (denosumab)	N/A	N/A

Abbreviations: g = gram; IMP = investigational medicinal product; IU = international units; N/A = not applicable; NIMP = non-investigational medicinal product; PFS = prefilled syringe; SC = subcutaneous.

IMP: Based on the randomisation schedule, either ENZ215 or EU-Prolia was administered to the participants.

Supplements: All the participants enrolled in the study were to take daily supplementation of elementary calcium and vitamin D until Month 12 or Month 18, as applicable. Compliance with daily calcium and vitamin D intake was monitored and assessed throughout the study.

During Vitamin D screening, if the 25 (OH) vitamin D level of a participant was < 20 ng/mL (< 50 mmol/L), replenishment of vitamin D deposits was attempted following sites standard of care (e.g., loading dose of vitamin D). After replenishment, vitamin D was retested to confirm value was within the eligibility range. The 25 (OH) vitamin D level of all participants was re-tested at Month 6.

Serum calcium level was tested locally in participants prior to dosing at Month 6 and Month 12. If hypocalcaemia was identified, the participant was not dosed until calcium levels were corrected.

Prohibited Medications:

- Patients were not allowed to take Xgeva during the study as ENZ215 and Prolia contain the same active ingredient as in Xgeva (denosumab).
- Use of the following medications was also prohibited during the study:
    - Any osteoporosis treatment (other than calcium and vitamin D supplements) such as:
        - Intravenous or oral bisphosphonates
        - Raloxifene or bazedoxifene
        - Teriparatide
        - Strontium ranelate or fluoride
        - Calcitonin or its derivates and calcimimetics (such as cinacalcet or etelcalcetide)
        - Parathyroid hormone or its derivatives, systemic hormone replacement therapy (oral or transdermal oestrogen; exceptionally, non-systemic vaginal oestrogen treatment is permitted), selective estrogen-receptor modulators, or tibolone
    - Drugs affecting bone metabolism:
        - ACTH
        - Anabolic steroids
        - Androgens
        - Anti-epileptics (except for benzodiazepines and pregabalin)
        - Aromatase inhibitors
        - Barbiturates
        - Calcimimetics
        - Systemic glucocorticoids (Topical and inhaled glucocorticosteroids are allowed).
        - Growth hormone-releasing hormone
        - GnRH agonists

- Heparin (including unfractionated heparin and low molecular weight heparins)
- Immunosuppressants, e.g., cyclosporine A, tacrolimus, methotrexate
- Lithium
- Protease inhibitors
- Systemic ketoconazole
- Tamoxifen
- Thiazolidinediones
- Warfarin
- Antidepressants such as selective serotonin reuptake inhibitors antipsychotics

If, in addition to the above, any medication(s) in the opinion of the Investigator was likely to affect the study outcome, the use of these medications was to be prohibited.

Invasive dental procedures (e.g., dental implants or oral surgery) and major surgeries or bone surgeries (unless required for AE/SAE management) should be avoided during the study period as far as possible.

- **Objectives and endpoints**

Table 19. Objectives and Endpoints

Objectives	**Endpoints**
Co-primary Efficacy Objectives	**Co-primary Efficacy Endpoints**
• To evaluate the efficacy of ENZ215 when compared to Prolia in participants with postmenopausal osteoporosis, in terms of change in BMD at the lumbar spine from baseline to Month 12	• Percentage change in BMD at lumbar spine (L1-L4 region) measured by DXA from baseline to Month 12
• To compare the AUEC of sCTX levels from baseline to Month 6	• AUEC of sCTX over the initial six months (from Day 1 pre-dose to Month 6 pre-dose)
Secondary Efficacy Objectives	**Secondary Efficacy Endpoints**
• To compare the change in sP1NP levels from baseline to Month 6	• Percentage change in sP1NP concentrations from baseline to Month 1, Month 3 and Month 6
• To compare the change in BMD at lumbar spine from baseline to Month 6	• Percentage change in BMD at lumbar spine measured by DXA from baseline to Month 6
• To compare the change in BMD at total hip and femoral neck from baseline to Month 6 and Month 12	• Percentage change in BMD at total hip and femoral neck measured by DXA from baseline to Month 6 and Month 12
Secondary Safety Objectives	**Secondary Safety Endpoints**

• To compare the immunogenicity potential of ENZ215 and Prolia	• ADAs incidence at baseline (Day 1) and Months 1, 3, 6, 9 and 12 and during open-label switch-over period, i.e. Months 15 and 18
• To compare the safety and tolerability of ENZ215 and Prolia	• Treatment-emergent serious and non-serious adverse events (TEAEs) during main treatment period and open-label switch-over period • Alteration in clinical laboratory parameters during main treatment period and open-label switch-over period
Secondary Pharmacokinetics Objective	**Secondary Pharmacokinetics Endpoint**
• To compare the pharmacokinetics of ENZ215 and Prolia	1. PK Parameters (Cmax, Tmax, partial AUC(0-1M), AUC(0-6M)) of denosumab measured at baseline (Day 1), Day 8, Day 15, Month 1, Month 3, Month 6 (prior to second dose) and Month 12

Abbreviations: ADA = anti-denosumab antibody; AUC = area under the concentration time curve; AUEC = area under the effect curve; BMD = bone mineral density; Cmax = maximum drug concentration; DXA = dual-energy X-ray absorptiometry; PK = pharmacokinetic; sCTX = serum C-telopeptide of type-1 collagen; sP1NP = serum procollagen type 1 N-terminal propeptide; TEAE = treatment-emergent adverse event; Tmax = time to peak drug concentration.

Co-primary endpoints

The co-primary efficacy endpoints were percentage change in BMD at the lumbar spine (L1-L4 region) from baseline to Month 12 measured by DXA and AUEC of sCTX level from baseline to Month 6.

Adoption of sCTX level as a co-primary endpoint was based on the recommendation from EMA during follow-up scientific advice. While the bone turnover markers are less universally validated endpoints for fracture risk than BMD, they are more sensitive measures of physiological effect. Baseline-normalised serum CTX seems to be the most sensitive PD endpoint to detect potential differences between biosimilar denosumab and the reference product, being an accepted marker for treatment effects from osteoporosis therapies. Therefore, CTX (at month 6) was adopted as the co-primary endpoint.

In clinical terms, the primary goal of osteoporosis therapy is to reduce fracture risk. BMD assessed by DXA remains the most widely utilised measure to identify patients at risk for fracture (Austin et. al. 2012). Since there is a strong association between increased BMD and decreased fracture (Black et al 2020), measured BMD changes underestimate improvements in bone strength but may nonetheless be proportional to reductions in fracture risk. As long as anti-fracture efficacy is roughly proportional to changes in BMD, such measurements will be clinically meaningful (Wasnich and Miller 2000).

Efficacy Assessments

- Bone density measurements (lumbar spine, hip and femoral neck) were performed, by Dual-energy X-ray Absorptiometry (DXA), at screening [Visit 1 (Day -35 to -1)], at Month 6 (Visit 7 [Day 180]), at Month 12 (Visit 9 [Day 360]) and at Month 18 (Visit 11 [Day 540]).
- Lateral thoracic and lumbar spine X-ray for fracture/vertebral abnormality assessment was performed at screening and Visit 9.

- Blood samples were collected for sCTX and serum procollagen type 1 N-terminal propeptide (sP1NP) at the designated time points as per the Schedule of Activities table as per study protocol.

For sCTX and sP1NP, blood samples were collected prior to IP administration (if applicable for the visit) at the same time (in the morning between 07:30 and 10:00 am) and after a minimum of 8 hours of fasting.

Equivalence Margin

For percentage change from baseline until month 12 in BMD at the lumbar spine the prespecified equivalence margins were [-1.45%, +1.45%].

For the area under the effect curve of the sCTX an acceptance range of 80-125% was proposed.

Estimands for the co-primary efficacy objective

Table 20. Summary of the primary and secondary estimands for estimating percentage change from baseline in BMD at Lumbar spine at month 12

Estimands	**Primary: "Treatment Policy" estimand (TPE)**	**Secondary: "Principal Stratum" estimand (PSE)**
Clinical Question of Interest	Do ENZ215 and Prolia® have a similar efficacy and a similar effect on BMD at the lumbar spine at month 12 in females with postmenopausal osteoporosis regardless of the ICEs occurring during the Double-Blind treatment period?	Do ENZ215 and Prolia® have a similar efficacy and a similar effect on BMD at the lumbar spine at month 12 in females with postmenopausal osteoporosis in the Principal Stratum of patients who would not experience any ICEs on either treatment arms?
Variable	Percent change in LS-BMD from Baseline to month 12, i.e., %CfB, is the primary study endpoint and is defined as: %CfB = (Post Baseline – Baseline) / Baseline * 100	
Treatments	Test product: ENZ215 subcutaneous injection 60 mg Reference product: Prolia® subcutaneous injection 60 mg	
Study Population	Females with postmenopausal osteoporosis as defined in detail in eligibility criteria (ITT Set)	The principal stratum of females with postmenopausal osteoporosis who would not experience any ICEs on either treatment (PP Set)
Intercurrent Events	**Strategy for Primary Estimand**	**Strategy for Secondary Estimand**
ICE1: Significant BMD assessments delays for more than 35 days at visit 9 (M12)	ICE1: Treatment policy strategy (All obtained data points will be included in the analysis, in line with the ITT principle.)	Principal stratum causal estimand strategy will be used: Only patients who would not experience either ICE if exposed to either treatment are relevant to the clinical question.

ICE2: The patient received other medication alongside the IP, which affects the primary variable (prohibited medications)	ICE2: Composite variable strategy (Composite variable strategy will be applied: Intercurrent event is considered to be informative about the outcome, so that the responses obtained after ICE occurrence will be imputed under the null hypothesis. In other words, responses obtained after ICE occurrence will be imputed with multiple imputation techniques so that outcomes observed after ICE2 occurrence will be modelled under the null hypothesis.)	To control the validity of the estimand dropout and ICE rates and reasons will be monitored.
Study Population	Females with postmenopausal osteoporosis.	The principal stratum of females with postmenopausal osteoporosis who would not experience any ICEs on either treatment.
Population-level Summary	Difference of means between the test and reference arms in percentage change from baseline LS-BMD: $\delta = \mu_{ENZ215} - \mu_{Prolia}$ μ_{ENZ215}: LS-BMD mean %CfB in ENZ215 study arm μ_{Prolia}: LS-BMD mean %CfB in Prolia® study arm	

No estimands were defined for the second co-primary endpoint AUEC0-6months or other secondary objectives/endpoints.

Study assessments

Efficacy assessment - Bone Mineral Density

BMD: DXA Spine and hip and femoral neck: All patients did undergo BMD assessments of the lumbar spine, total hip, and femoral neck performed by DXA at baseline (screening/Day 1), Month 6, and Month 12.

DXA should be done and submitted as early as possible during the screening period to allow for enough time in case a repeat exam is requested by Medical Imaging. In addition, as far as possible, DXA should be done during the applicable study visits and before dosing. Exceptionally, in case DXA cannot be done before dosing and/or on the same day as the rest of the visit procedures at Month 6 and Month 12, it can be performed within the allowed window period of ±7 days.

The results will be evaluated by a central assessor. The same DXA machine will be used for all study procedures for a particular patient. Detailed instructions on DXA scan acquisition are provided separately in the Image Acquisition Guideline.

Instrument quality control (IQC) is carried out in order to allow for correction of any DXA instrument calibration shifts or drift that may occur during the course of the study. Local spine phantom data will be obtained regularly from each DXA machine in the course of the study and this will be analyzed by cumulative sum analysis (CUSUM; analysis done by the software) to identify significant shifts or drifts in calibration and

generate IQC corrections accordingly. These IQC corrections can then be applied to patient DXA BMD results accordingly. IQC BMD corrections are scanner and time-specific and are applied where necessary to the final DXA data set. Further information is provided in the BMD charter document.

- **Sample size**

The sample size for study was computed to demonstrate equivalence of ENZ215 and Prolia in the percent change from baseline in LS-BMD at 12 months. The equivalence margin was pre-defined at ±1.45% as discussed above. Assuming a standard deviation (SD) of 4.16%, the study would have 90% power to demonstrate equivalence at the (2-sided) 2.5% level of significance with 214 evaluable patients in each treatment group. Allowing for a 15% dropout rate, 504 patients (252 per treatment group) were required to be randomised in the study.

Considering the PD co-primary endpoint (percentage change from baseline in AUEC $sCTX_{0\text{-}6m}$), healthy volunteer data were used for the sample size calculations due to the lack of information on AUEC $sCTX_{0\text{-}6m}$ derived from the patient population. The expected variability to the proposed PD endpoint was considered to be significantly lower than that of the proposed efficacy endpoint (in the NCT2053753 study, the inter-subject CV of AUEC $sCTX_{0\text{-}m6}$ was approximately 28% [NCT2053753 study results]).

The correlation between the sCTX and LS-BMD was assumed to be zero. Most likely there was a correlation between percentage change from baseline in LS-BMD and log (AUEC). It was difficult to estimate the value a-priori and the correlation of zero provided a conservative estimate of the power.

A total sample size of 428 patients had >99.9% power for the co-primary endpoint percentage change from baseline in AUEC sCTX0-6m considering the CV of 28%. The overall power of the study was approximately 90% to succeed on both the equivalence tests for co-primary endpoints.

- **Randomisation and Blinding (masking)**

Randomisation: Patients were randomized in a 1:1 ratio to receive either ENZ215 or Prolia (60 mg SC injection) at baseline and Month 6. A subset of 120 patients initially randomized to Prolia arm was re-randomized in a 1:1 ratio (i.e. 60 patients in each arm) in order to have 100 evaluable patients (i.e. 50 patients in each arm) at Month 12.

Patient allocation was stratified by age (≥ 55 to < 70 years and ≥ 70 to ≤ 85 years) and based on prior use of bisphosphonate.

All patients were to be centrally randomized using an Interactive Response Technology (IRT). Each patient was to be assigned a unique number (randomization number) that encodes the patient's assignment to one of the 2 arms of the study, according to the randomization schedule. The randomization schedule was generated by the IRT vendor.

Blinding: This is a double-blind study (until Month 12) in which patients and Investigators were blinded to study intervention. The IRT was to be programmed with blind-breaking instructions. In case of an emergency, the Investigator had the sole responsibility for determining if unblinding of a patient's study intervention assignment was warranted. Patient safety always had to be the first consideration in making such a determination. If the Investigator decided that unblinding was warranted, the Investigator was to make every effort to contact the Sponsor prior to unblinding a patient's study intervention assignment unless this could delay emergency treatment for the patient. If a patient's study intervention assignment was unblinded, the Sponsor had to be notified within 24 hours of this occurrence. The date and reason for the unblinding was to be recorded.

Data were to be frozen at patient level before patient transition from the double-blind treatment period to the open-label extension study. Further details of transition of patients to the open-label extension study were to be outlined in the Data Management Plan.

- **Statistical methods**

Analysis Set

The study analysis sets were defined as follows:

Screened Set: The screened set consisted of all participants who signed informed consent.

Intent-to-Treat (ITT) Set**:** The ITT analysis set consisted of all randomised participants who received at least one dose of study intervention in the double-blind treatment period. In the ITT analysis set, treatment was assigned based on the study intervention to which participants were randomised, regardless of which treatment they actually received.

Modified ITT (mITT) Set**:** The mITT analysis set consisted of all ITT participants who had baseline assessment and post-baseline LS-BMD value.

Per-Protocol (PP) Set**:** The PP set was a subset of the ITT set with the LS-BMD assessments at baseline and Month 12 and consisted of all participants who had no major protocol deviations which affected LS-BMD, received the study intervention at baseline and Month 6, and had baseline and Month 12 LS-BMD data.

Safety Set**:** The safety set included all randomised participants who received at least one dose of study intervention. In the safety set, treatment was assigned based on the actual treatment that participants received.

The ITT set will be the primary analysis set for all efficacy data related to BMD data. Efficacy data will also be analysed for the mITT and PP set to corroborate the results of the ITT analysis. All safety data will be analysed for the safety set.

Upon database release, protocol deviation and analysis set outputs will be produced and will be sent to Sponsor for review. An analysis set classification meeting will be arranged to discuss the outputs and to decide which patients and/or patient data will be excluded from certain analyses.

Decisions made regarding the exclusion of patients and/or patient data from analyses will be made prior to database lock and will be documented and approved by Sponsor.

Co-Primary Endpoints Analysis (Efficacy):

Primary estimand ('treatment policy estimand')

The mean percentage change from baseline to Month 12 in LS-BMD was to be analysed using an analysis of covariance (ANCOVA) model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates on the ITT set.

Missing data without experiencing ICE2 was assumed to be MCAR and was not to be imputed. BMD values at 12 months which were assessed with more than 35 days of delay (intercurrent event 1) were to be included in the analysis as observed in line with the intention-to-treat principle. For patients who used prohibited medication (intercurrent event 2), data in the Prolia arm was imputed under the assumption of missing at random, whereas data in the ENZ215 arm was imputed assuming missing not at random, by first imputing under MAR and then shifting by -1.45%.

The test product and reference product were to be declared comparable if the 95% CI of the treatment difference for the mean percentage change from baseline to Month 12 in LS-BMD was within the pre-defined equivalence margin of ± 1.45.

As a sensitivity analysis, an analysis similar to the primary analysis was to be performed but imputing missing data in patients not using prohibited medication under the assumption of MAR. Additionally, this sensitivity analysis and the primary analysis were repeated on the mITT and PP population.

Upon request a modified version of the primary analysis was provided where treatment discontinuation and lumbar spine-related adverse events including vertebral fractures, lumbar spine surgery and other bone-involving AEs in the lumbar region were considered as intercurrent events in addition to delay in month 12 LS-BMD assessment and use of prohibited medications. All available data after intercurrent events were included in the analysis in an attempt to implement the treatment policy strategy. Missing values after intercurrent events were imputed by drawing from a normal distribution centered around 0.

Secondary estimand ('principal stratum estimand')

The secondary estimand was to be primarily analysed by performing an ANCOVA model with treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as independent variables on the per-protocol set excluding patients who experienced intercurrent events. The same similarity criterion was applied for this analysis as for the primary estimand.

As a sensitivity analysis, the bias obtained by using the per-protocol set instead of the not identifiable subset of patients who would be compliant with study under both ENZ215 and Prolia was investigated (*Lou, Y., Jones, M. P., & Sun, W. (2018)).*

Estimand based on hypothetical strategy

In addition to the primary estimand (based on treatment policy strategy) and secondary estimand (based on principle stratum strategy), an estimand applying a hypothetical strategy for the two intercurrent events BMD assessment delays and prohibited medication was investigated using an MMRM approach. Data points captured after intercurrent events were to be left out from the analysis. The MMRM was to include treatment, age group, previous use of bisphosphonates, baseline BMD values, visit (month 6 or month 12) and an interaction between visit and treatment as independent variables. Missing data was not to be imputed for the MMRM approach.

Upon request, the Applicant provided a revised analysis taking into account treatment discontinuation and lumbar spine-related adverse events as intercurrent events in addition to delay in BMD assessment and use of prohibited medications.

Analysis of Secondary Efficacy Endpoints

Descriptive statistics were to be used for the analysis of secondary endpoints.

Error probabilities, adjustment for multiplicity and interim analyses

There was no interim analysis planned in this study. It was planned to perform two analyses and to prepare two CSRs: one at Month 12 primary endpoint analysis and second one at the final database lock at end of open-label phase, Month 18. However, as data for Month 12 and Month 18 were available at the time of report, final CSR was prepared for the study.

Generally, no adjustment for multiplicity was conducted in this trial.

Changes from protocol-specified analyses

There were no relevant changes from protocol-specified analyses.

Results

- **Participant flow**

Date of First Enrollment: 04 Jul 2022

Date of Last Participant Completed**:** 18 Jul 2024

Database lock: 04 Sep 2024

Table 21. Total number of sites and subjects enrolled per country

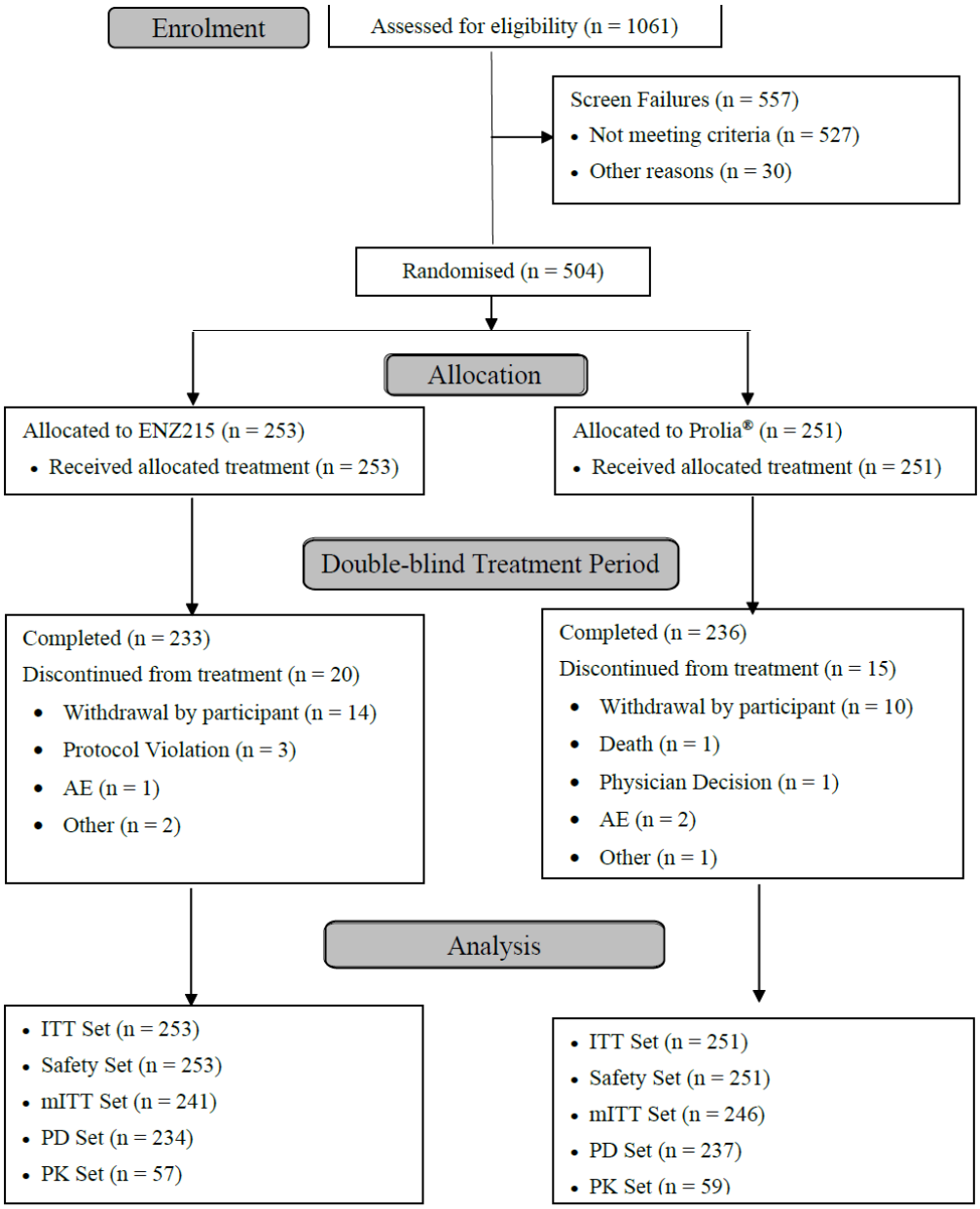
Country	Total number of sites enrolling patients	Total number of subjects enrolled
Bulgaria	4	54
Czech Republic	6	134
Poland	22	230
Serbia	3	39
Spain	2	2
Lithuania	7	45

- **Recruitment**

Disposition of Participants

Double-blind treatment period:

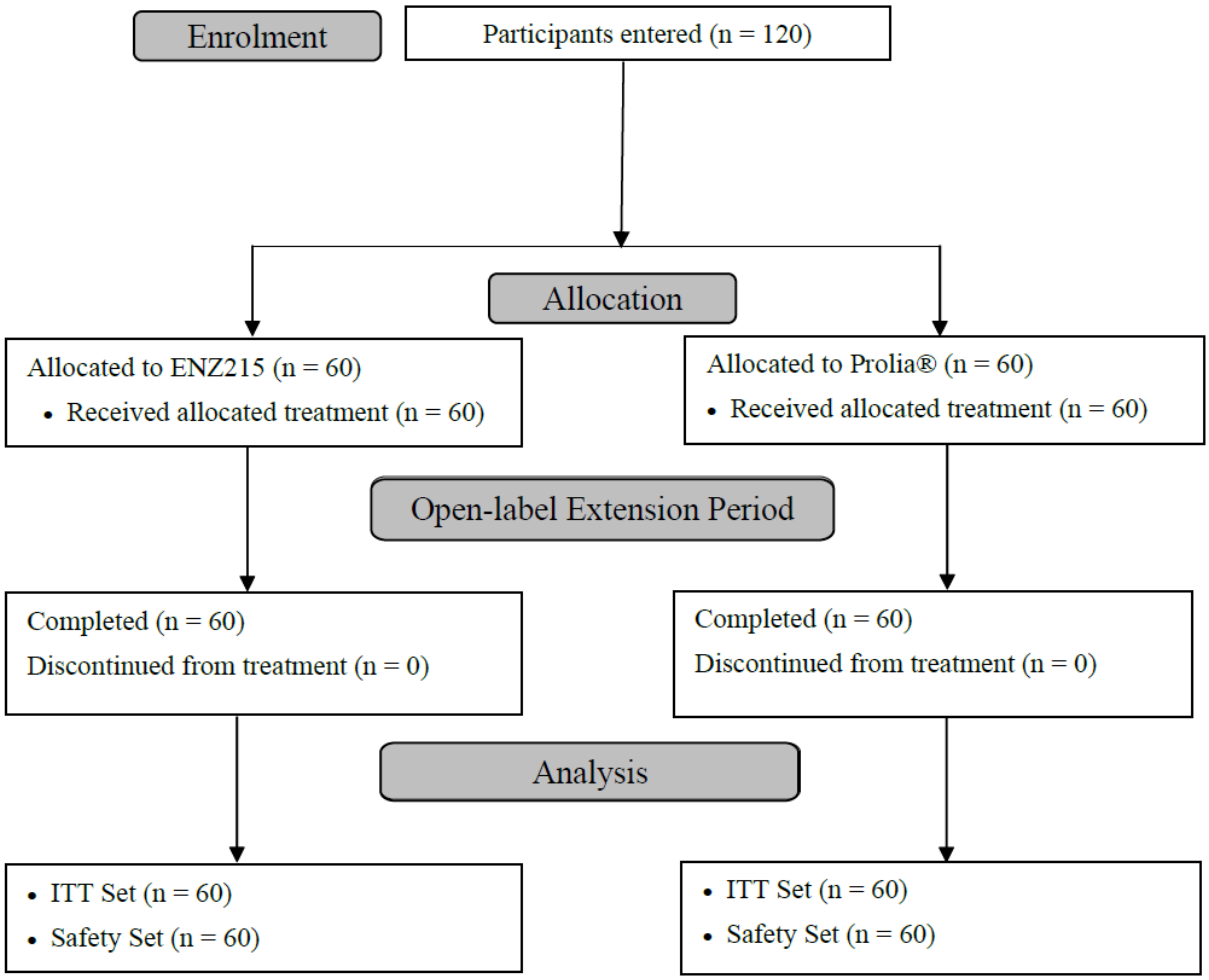
Figure 8. Summary of Participant Disposition and Analysis Sets (CONSORT Diagram) - Double-blind Treatment Period



Abbreviations: AE = adverse event; CONSORT = consolidated standards of reporting trials; ITT = intent-to-treat; mITT = modified intent-to-treat; PD = pharmacodynamics; PK = pharmacokinetics.

Open-label extension period: All 120 participants (100.0%) who entered the open-label extension period received one injection of study treatment during the open-label extension period.

Figure 9. Summary of Participant Disposition and Analysis Sets (CONSORT Diagram) - Open-label Extension Period



Abbreviations: CONSORT = consolidated standards of reporting trials; ITT = intent-to-treat.

Protocol Deviations

Table 14.1.2.1a: Protocol Deviations (PDs) with inclusion of corrected PDs (ITT set)

Period: Double blind

	Protocol Deviation Category	ENZ215 (N = 253) n (%)	Prolia® (N = 251) n (%)	Overall (N = 504) n (%)
Patients with protocol deviation		178 (70.4)	170 (67.7)	348 (69.0)
AE SAE	Major	0	1 (0.4)	1 (0.2)
Disallowed Medications	Major	6 (2.4)	3 (1.2)	9 (1.8)
Disallowed Medications	Minor	10 (4.0)	6 (2.4)	16 (3.2)
Inc/Excl Criteria	Major	2 (0.8)	0	2 (0.4)
Informed Consent	Major	5 (2.0)	6 (2.4)	11 (2.2)
IP Admin/Study Treat	Minor	38 (15.0)	41 (16.3)	79 (15.7)
Procedures/Tests	Major	3 (1.2)	3 (1.2)	6 (1.2)
Procedures/Tests	Minor	116 (45.8)	99 (39.4)	215 (42.7)
Visit Schedule	Major	6 (2.4)	5 (2.0)	11 (2.2)
Visit Schedule	Minor	77 (30.4)	85 (33.9)	162 (32.1)

Period: Open label

	Protocol Deviation Category	ENZ215 (N = 60) n (%)	Prolia® (N = 60) n (%)	Overall (N = 120) n (%)
Patients with protocol deviation		25 (41.7)	23 (38.3)	48 (40.0)
Disallowed Medications	Minor	1 (1.7)	2 (3.3)	3 (2.5)
IP Admin/Study Treat	Minor	5 (8.3)	2 (3.3)	7 (5.8)
Procedures/Tests	Minor	9 (15.0)	6 (10.0)	15 (12.5)
Visit Schedule	Minor	14 (23.3)	15 (25.0)	29 (24.2)

ITT = Intent-To-Treat Population Flag; N = The total number of patients in ITT Set; n= Number of patients with non-missing data within the specific category.

Please refer to section "Baseline data" subsection "Concomitant medication" below for the 9 participants with protocol deviations "disallowed medications" during the double-blind period.

- **Conduct of the study**

The original protocol (version 1.0) of the DEN2 study is dated 22 Oct 2021. The protocol was amended twice *before* the date of first enrolment (which was on 04 Jul 2022) to version 1.1 (16 Dec 2021) and version 2.0 (08 Apr 2022). *After* enrolment initiation, the protocol was amended once to version 3.0 (16 Feb 2023) to add clarifications and update typo errors noted in earlier version of the document.

- **Baseline data**

Table 22. Demography and Other Baseline Characteristics (ITT Set)

Demographic Variable	ENZ215	Prolia®	Overall
Double-blind treatment period	**(N = 253)**	**(N = 251)**	**(N = 504)**
Age (years)			
n	253	251	504
Mean	66.3	66.1	66.2
SD	6.56	6.35	6.45
Minimum	55	55	55
Median	66.0	66.0	66.0
Maximum	84	82	84
Age Group, n (%)			
n	253	251	504
≥ 55 to < 70 years	171 (67.6)	171 (68.1)	342 (67.9)
≥ 70 to ≤ 85 years	82 (32.4)	80 (31.9)	162 (32.1)
Prior use of Bisphosphonate, n (%)			
Yes	44 (17.4)	43 (17.1)	87 (17.3)
No	209 (82.6)	208 (82.9)	417 (82.7)
Sex			
Female	253 (100)	251 (100)	504 (100)
Years since menopause			
n	253	251	504
Mean	17.2	16.8	17.0
SD	7.42	7.22	7.31
Minimum	5	5	5
Median	17.0	17.0	17.0
Maximum	40	42	42
Ethnicity			
Hispanic or Latino	0	1 (0.4)	1 (0.2)
Not Hispanic or Latino	253 (100)	250 (99.6)	503 (99.8)
Not Stated	0	0	0

Demographic Variable	ENZ215	Prolia®	Overall
Unknown	0	0	0
Race			
n	253	251	504
American Indian or Alaska Native	0	0	0
Asian	0	0	0
Black or African American	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
White	253 (100)	251 (100)	504 (100)
Other	0	0	0
Not Reported	0	0	0
Weight (kg)			
n	253	251	504
Mean	63.33	64.35	63.84
SD	8.613	9.444	9.042
Minimum	50.0	50.0	50.0
Median	62.00	63.00	62.65
Maximum	90.0	90.0	90.0
Height (cm)			
n	253	251	504
Mean	159.17	159.10	159.13
SD	6.281	6.344	6.306
Minimum	140.0	143.0	140.0
Median	159.50	158.50	159.00
Maximum	174.0	176.0	176.0
BMI (kg/m^2)			
n	253	251	504
Mean	25.03	25.48	25.26
SD	3.412	3.874	3.652
Minimum	17.8	18.1	17.8
Median	24.70	24.70	24.70
Maximum	36.1	37.5	37.5
Open-label extension period	**(N = 60)**	**(N = 60)**	**(N = 120)**
Age (years)			
n	60	60	120
Mean	65.8	66.4	66.1
SD	6.97	6.10	6.53
Minimum	55	56	55
Median	67.0	66.0	66.0
Maximum	80	82	82

Demographic Variable	ENZ215	Prolia®	Overall
Age group, n(%)			
n	60	60	120
≥ 55 to < 70 years	40 (66.7)	41 (68.3)	81 (67.5)
≥ 70 to ≤ 85 years	20 (33.3)	19 (31.7)	39 (32.5)
Prior use of Bisphosphonate, n (%)			
Yes	13 (21.7)	13 (21.7)	26 (21.7)
No	47 (78.3)	47 (78.3)	94 (78.3)
Sex			
Female	60 (100)	60 (100)	120 (100)
Years since menopause			
n	60	60	120
Mean	16.6	17.1	16.8
SD	7.40	7.67	7.51
Minimum	5	5	5
Median	17.0	17.0	17.0
Maximum	33	42	42
Ethnicity			
Hispanic or Latino	0	1 (1.7)	1 (0.8)
Not Hispanic or Latino	60 (100)	59 (98.3)	119 (99.2)
Not Stated	0	0	0
Unknown	0	0	0
Race			
n	60	60	120
American Indian or Alaska Native	0	0	0
Asian	0	0	0
Black or African American	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
White	60 (100)	60 (100)	120 (100)
Other	0	0	0
Not Reported	0	0	0
Weight (kg)			
n	60	60	120
Mean	64.88	63.56	64.22
SD	9.357	9.058	9.194
Minimum	50.0	50.3	50.0
Median	63.65	62.25	63.20
Maximum	89.0	84.5	89.0
Height (cm)			
n	60	60	120

Demographic Variable	ENZ215	Prolia®	Overall
Mean	160.20	158.58	159.39
SD	6.560	6.128	6.373
Minimum	146.5	146.0	146.0
Median	159.75	157.75	159.00
Maximum	176.0	173.0	176.0
BMI (kg/m^2)			
n	60	60	120
Mean	25.35	25.36	25.35
SD	3.899	3.863	3.865
Minimum	18.6	18.1	18.1
Median	24.65	24.95	24.75
Maximum	36.6	33.4	36.6

Abbreviations: BMI = body mass index; ITT = intent-to-treat; N = total number of participants in ITT Set; n = number of participants with non-missing data within the specific category; SD = standard deviation.

Percentages are based on the number of participants providing data for the respective category.

Source: Table 14.1.4.1.

Baseline Disease Characteristics

Table 23. Fracture or X-ray Finding History (ITT Set)

Treatment Group	ENZ215 (N = 253)	Prolia® (N = 251)	Overall (N = 504)
Number (%) of Participants	n (%)	n (%)	n (%)
Any fracture history?			
Yes	118 (46.6)	110 (43.8)	228 (45.2)
No	135 (53.4)	141 (56.2)	276 (54.8)
Fracture history			
Acetabulum fracture	0	2 (0.8)	2 (0.4)
Ankle fracture	17 (6.7)	4 (1.6)	21 (4.2)
Cervical vertebral fracture	2 (0.8)	1 (0.4)	3 (0.6)
Clavicle fracture	3 (1.2)	3 (1.2)	6 (1.2)
Femur fracture	0	1 (0.4)	1 (0.2)
Foot fracture	13 (5.1)	10 (4.0)	23 (4.6)
Forearm fracture	14 (5.5)	13 (5.2)	27 (5.4)
Fracture	1 (0.4)	0	1 (0.2)
Hand fracture	10 (4.0)	8 (3.2)	18 (3.6)
Humerus fracture	10 (4.0)	14 (5.6)	24 (4.8)
Jaw fracture	1 (0.4)	0	1 (0.2)
Joint dislocation	1 (0.4)	0	1 (0.2)
Lower limb fracture	2 (0.8)	0	2 (0.4)
Lumbar vertebral fracture	12 (4.7)	12 (4.8)	24 (4.8)
Open reduction of fracture	1 (0.4)	0	1 (0.2)
Osteoporotic fracture	0	1 (0.4)	1 (0.2)
Patella fracture	1 (0.4)	3 (1.2)	4 (0.8)
Pelvic fracture	2 (0.8)	1 (0.4)	3 (0.6)
Radius fracture	13 (5.1)	7 (2.8)	20 (4.0)
Rib fracture	4 (1.6)	4 (1.6)	8 (1.6)
Scapula fracture	1 (0.4)	0	1 (0.2)
Shoulder fracture	0	2 (0.8)	2 (0.4)
Spinal compression fracture	1 (0.4)	0	1 (0.2)
Spinal fracture	1 (0.4)	3 (1.2)	4 (0.8)
Sternal fracture	0	1 (0.4)	1 (0.2)
Thoracic vertebral fracture	21 (8.3)	29 (11.6)	50 (9.9)
Tibia fracture	1 (0.4)	1 (0.4)	2 (0.4)
Traumatic fracture	0	1 (0.4)	1 (0.2)
Ulna fracture	1 (0.4)	3 (1.2)	4 (0.8)
Upper limb fracture	4 (1.6)	8 (3.2)	12 (2.4)

Treatment Group	ENZ215 (N = 253)	Prolia® (N = 251)	Overall (N = 504)
Wrist fracture	26 (10.3)	18 (7.2)	44 (8.7)
Any Lateral Lumbar Spine X-Ray performed?			
Yes	253 (100)	251 (100)	504 (100)
No	0	0	0
X-Ray results			
Normal	149 (58.9)	160 (63.7)	309 (61.3)
Abnormal, not clinically significant	104 (41.1)	91 (36.3)	195 (38.7)
Abnormal, clinically significant	0	0	0
Vertebral fractures	30 (11.9)	40 (15.9)	70 (13.9)
Cervical vertebral fracture	2 (0.8)	1 (0.4)	3 (0.6)
Lumbar vertebral fracture	12 (4.7)	12 (4.8)	24 (4.8)
Spinal compression fracture	1 (0.4)	0	1 (0.2)
Spinal fracture	1 (0.4)	3 (1.2)	4 (0.8)
Thoracic vertebral fracture	21 (8.3)	29 (11.6)	50 (9.9)

Abbreviations: ITT = intent-to-treat; N = total number of participants in ITT Set; n = number of participants with non-missing data within the specific category.
Fracture and X-Ray finding history details are considered from screening visit data.
Source: Table 14.1.5.1.

Medical History and Concurrent Illnesses

Most of the participants reported medical history belonging to the Musculoskeletal and Connective Tissue Disorders SOC (293 participants overall [58.1%]) and the Vascular Disorders SOC (245 participants overall [48.6%]) (Table 14.1.6.1). At the PT level, the most frequently reported medical histories were hypertension (99 participants [39.1%] in the ENZ215 group and 93 participants [37.1%] in the Prolia group), osteoarthritis (82 participants [32.4%] in the ENZ215 group and 74 participants [29.5%] in the Prolia group) and spinal osteoarthritis (57 participants [22.5%] in the ENZ215 group and 51 participants [20.3%] in the Prolia group).

Prior and Concomitant Therapy

Prior medications: A total of 398 participants (79.0%) had at least one prior medication. The most common (≥ 5% of participants overall) prior medications by ATC level 3 were:

- Vitamin A and D, including combinations of the two (282 participants overall [56.0%]: 149 participants [58.9%] in the ENZ215 group; 133 participants [53.0%] in the Prolia group)
- Calcium (218 participants overall [43.3%]: 105 participants [41.5%] in the ENZ215 group; 113 participants [45.0%] in the Prolia group)
- Viral vaccines (133 participants [26.4%]: 64 participants [25.3%] in the ENZ215 group; 69 participants [27.5%] in the Prolia group)
- Drugs affecting bone structure and mineralisation (87 participants overall [17.3%]: 45 participants [17.8%] in the ENZ215 group; 42 participants [16.7%] in the Prolia group).

Regarding COVID-19 vaccination, 113 participants overall (22.4%) received tozinameran, 15 participants (3.0%) received COVID-19 vaccine Nrvv Ad (CHADOX1 NCOV-19), 14 participants (2.8%) received

elasomeran, seven participants (1.4%) received unspecified COVID-19 vaccine and four participants (0.8%) received COVID-19 vaccine Nrvv Ad26 (JNJ 78436735).

All participants with at least one prior medication under the ATC class "Drugs affecting bone structure and mineralisation" had discontinued the medication before study entry per study protocol requirements.

Concomitant medications:

Double-blind treatment period: A total of 282 participants overall (56.0%) had at least one concomitant medication. The most common (≥ 5% of participants overall) concomitant medications by ATC level 3 were:

- Anti-inflammatory and anti-rheumatic products, non-steroids (77 participants overall [15.3%]: 46 participants [18.2%] in the ENZ215 group; 31 participants [12.4%] in the Prolia group)
- Other analgesics and antipyretics (71 participants overall [14.1%]: 36 participants [14.2%] in the ENZ215 group; 35 participants [13.9%] in the Prolia group)
- Beta-lactam antibacterials, penicillins (38 participants [7.5%]: 19 participants [7.5%] in the ENZ215 group; 19 participants [7.6%] in the Prolia group)
- Drugs for peptic ulcer and gastro-oesophageal reflux disease (37 participants [7.3%]: 23 participants [9.1%] in the ENZ215 group; 14 participants [5.6%] in the Prolia group)
- Macrolides, lincosamides and streptogramins (37 participants [7.3%]: 18 participants [7.1%] in the ENZ215 group; 19 participants [7.6%] in the Prolia group)
- Expectorants, excl. combinations with cough suppressants (33 participants [6.5%]: 17 participants [6.7%] in the ENZ215 group; 16 participants [6.4%] in the Prolia group)
- Viral vaccines (33 participants [6.5%]: 17 participants [6.7%] in the ENZ215 group; 16 participants [6.4%] in the Prolia group)
- Other antibacterials (28 participants [5.6%]: 14 participants [5.5%] in the ENZ215 group; 14 participants [5.6%] in the Prolia group)
- Other beta-lactam antibacterials (28 participants [5.6%]: 16 participants [6.3%] in the ENZ215 group; 12 participants [4.8%] in the Prolia group)

Major protocol deviations related to disallowed medications were reported in nine participants overall (1.8%): six participants (2.4%) in the ENZ215 group and three participants (1.2%) in the Prolia group. In the ENZ215 group, disallowed medications included enoxaparin sodium, trazodone hydrochloride, sertraline, citalopram, tiapride and warfarin (one participant each). All participants were excluded from both the PP and PD Sets, except for the participants who took enoxaparin sodium and warfarin who were only excluded from the PP Set. In the Prolia group, disallowed medications included enoxaparin sodium (two participants), pioglitazone and prednisone (one participant). The participant taking pioglitazone and prednisone was excluded from the PP set; the two participants taking enoxaparin sodium were excluded from both the PP and PD Sets.

Overall, minor protocol deviations related to disallowed medications were reported in 14 participants (2.8%) during the double-blind period.

Open-label extension period: A total of 39 participants overall (32.5%) had at least one concomitant medication. The most common (≥ 5% of participants overall) concomitant medications by ATC level 3 were other analgesics and antipyretics (11 participants overall [9.2%]: five participants [8.3%] in the ENZ215 group; 6 participants [10.0%] in the Prolia group).

There were no major protocol deviations related to disallowed medications during the open-label period. Overall, minor protocol deviations related to disallowed medications were reported in five participants (4.2%).

- **Numbers analysed**

Table 24. Analysis Sets (Screened Set)

	ENZ215	Prolia	Overall
Number (%) of Participants	**n (%)**	**n (%)**	**n (%)**
Double-blind treatment period			
Screened Set			1,061
Intent-to Treat (ITT) Set	253 (100)	251 (100)	504 (100)
Modified ITT (mITT) Set	241 (95.3)	246 (98.0)	487 (96.6)
Per-Protocol (PP) Set	230 (90.9)	233 (92.8)	463 (91.9)
Safety Set	253 (100)	251 (100)	504 (100)
PD Set	234 (92.5)	237 (94.4)	471 (93.5)
PK Set	57 (22.5)	59 (23.5)	116 (23.0)
Open-label extension period			
Intent-to Treat (ITT) Set	60 (100.0)	60 (100.0)	120 (100.0)
Safety Set	60 (100.0)	60 (100.0)	120 (100.0)

Abbreviations: AUC = area under the concentration time curve; AUEC = area under the effect curve; Cmax = maximum drug concentration; ITT = intent-to-treat; ITTFL = intent-to-treat population flag; LS-BMD = least square bone mineral density; mITT = modified intent-to-treat; n = number of participants screened per category; PD = pharmacodynamics; PK = pharmacokinetics; PP = per-protocol; sCTX = serum C-telopeptide of type 1 collagen; sP1NP = procollagen type 1 N-terminal propeptide.

The Screened Set will consist of all participants who have signed informed consent.

The ITT analysis set consists of all randomised participants who received at least one dose of study intervention. The mITT analysis set consists of all ITT participants who have baseline assessment and post-baseline assessment for primary efficacy endpoint.

The PP set is a subset of the ITT set with the LS-BMD assessments at baseline and Month 12 and consists of all participants who do not have any *major protocol deviations*, receive the study intervention at baseline and Month 6, and have baseline and Month 12 data.

The safety set includes all randomised participants who receive at least one dose of study intervention.

The PD set consists of all participants in the safety set whose sCTX values are available in order to calculate PD parameter AUEC values for primary analysis and do not have any *major protocol deviations which would affect sCTX or sP1NP measurement*.

The PK set is a subset of safety set with at least one evaluable PK endpoint (C_{max} or $AUC_{0\text{-}6M}$) and no *major protocol deviations affecting the PK parameters up to Month 12*.

For percentage calculations ITTFL populations were used.

Table 25. Protocol deviation leading to exclusion from different analysis sets (ITT Set)

Period: Double blind

	Protocol Deviation Category	Excluded from Analysis Set	ENZ215 (N = 253) n (%)	Prolia® (N = 251) n (%)	Overall (N = 504) n (%)
Patients with protocol deviation					
Disallowed Medications	Major	PD Set	4 (1.6)	2 (0.8)	6 (1.2)
Disallowed Medications	Major	PP Set	6 (2.4)	3 (1.2)	9 (1.8)
Inc/Excl Criteria	Major	PD Set	2 (0.8)	0	2 (0.4)
Inc/Excl Criteria	Major	PP Set	2 (0.8)	0	2 (0.4)
Informed Consent	Major	PK Set	5 (2.0)	6 (2.4)	11 (2.2)
Procedures/Tests	Major	PD Set	1 (0.4)	1 (0.4)	2 (0.4)
Procedures/Tests	Major	PK Set	1 (0.4)	1 (0.4)	2 (0.4)
Procedures/Tests	Minor	PK Set	33 (13.0)	27 (10.8)	60 (11.9)
Procedures/Tests	Minor	PP Set	1 (0.4)	0	1 (0.2)
Visit Schedule	Major	PD Set	2 (0.8)	4 (1.6)	6 (1.2)
Visit Schedule	Major	PK Set	1 (0.4)	0	1 (0.2)
Visit Schedule	Major	PP Set	4 (1.6)	2 (0.8)	6 (1.2)
Visit Schedule	Minor	PP Set	3 (1.2)	3 (1.2)	6 (1.2)

Period: Open label

Protocol Deviation Category	Excluded from Analysis Set	ENZ215 (N = 60) n (%)	Prolia® (N = 60) n (%)	Overall (N = 120) n (%)
No data to report				

ITT = Intent-To-Treat Population Flag; N = The total number of patients in ITT Set; n= Number of patients with non-missing data within the specific category.

PK Set/Sub-study: In the double-blind treatment period, minor protocol deviations related to procedures/tests resulted in the exclusion from the PK set of 60 participants overall (11.9%). Major protocol deviations related to informed consent resulted in the exclusion from the PK Set of 11 participants overall (2.2%). Note: In the PK sub-study, more than the protocol-defined set of 120 participants were enrolled which were excluded from the PK sub-study after collecting an initial one or two PK samples and thus were not included in PK analysis set. None of the participants were excluded from the different analysis sets in the open-label extension period.

- **Outcomes and estimation**

Study ALK22/ENZ215-DEN2 investigated two co-primary endpoints:

- Percentage change in BMD at lumbar spine (L1-L4 region) measured by DXA from baseline to Month 12 (discussed in this section)
- AUEC of sCTX over the initial six months (from Day 1 pre-dose to Month 6 pre-dose) (discussed in the PD section 3.3.1.2.2)

Co-Primary Efficacy Endpoint:

Percentage Change in BMD at Lumbar Spine (L1-L4 Region) Measured by DXA From Baseline to Month 12

The primary efficacy analysis was performed for the ITT set comparing percent change from baseline in lumbar spine BMD at Month 12 between ENZ215 and Prolia treatment groups.

At baseline (D1), the mean (SD) BMD was 0.7466 (0.0691) in the ENZ215 group and 0.7525 (0.0684) in the Prolia group.

Table 26. Percentage Change in BMD at Lumbar Spine (L1-L4 Region) Measured by DXA From Baseline to Month 12 to Assess Bioequivalence of ENZ215 Compared to Prolia - Primary Endpoint Analysis (ITT Set)

Treatment Group	N	Adjusted LS Mean (SE)	95% CI of adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference
ENZ215 (N = 253)	231	5.350 (0.3152)	4.7306; 5.9695	-0.183	-0.9044; 0.5380
Prolia (N = 251)	235	5.533 (0.3108)	4.9225; 6.1440		

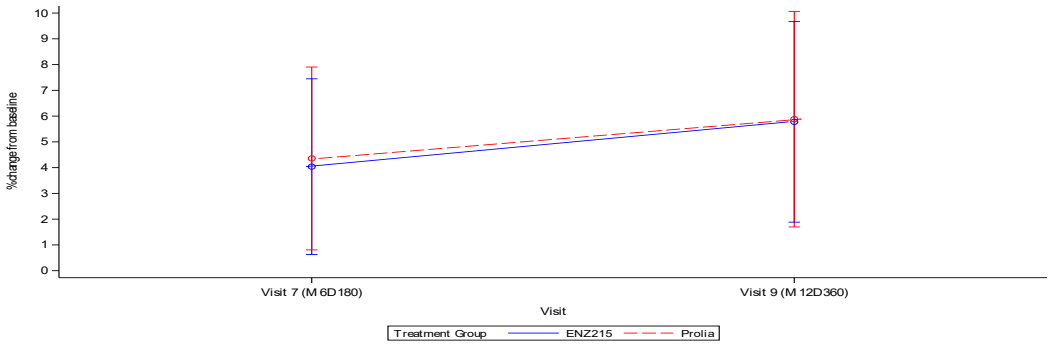
Abbreviations: ANCOVA = analysis of covariance; BMD = bone mineral density; CI = confidence interval; DXA = dual-energy X-ray absorptiometry; ITT = intent-to-treat; LS Mean = least square mean; N = total number of participants in ITT Set; n = number of participants with non-missing data within the specific category; SE = standard error.

Analysis was performed using an ANCOVA model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates.

Equivalence was established if 95% CIs of LS Mean difference lied between -1.45 and 1.45.

Source: ALK22/ENZ215-DEN2 CSR Table 14.2.1.2.

Figure 10. Mean (± SD) of Percentage Change from Baseline in BMD at Lumbar Spine (L1-L4 Region) Measured by DXA From Baseline Over Time by Treatment Group (ITT Set)



Abbreviations: BMD = bone mineral density; D = day; DXA = dual-energy X-ray absorptiometry; ITT = intent-to-treat; M = month; SD = standard deviation.
Source: Figure 14.2.1.2.

The number of participants with ICE in the ITT set is presented in the table below.

Table 27. Intercurrent Events (ITT Set)

Characteristics	ENZ215 (N = 253) n (%)	Prolia® (N = 251) n (%)	Overall (N = 504) n (%)
ICE1	6 (2.4)	6 (2.4)	12 (2.4)
ICE2	6 (2.4)	3 (1.2)	9 (1.8)

ITT = Intent-To-Treat Population Flag; ICE1 = Intercurrent Event 1; ICE2 = Intercurrent Event 2

N = The total number of patients in ITT Set; n = Number of patients with non-missing data within the specific category;

ICE1 = Significant BMD assessment delays for more than 35 days at visit 9 (M12)

ICE2 = The patient received other medication alongside the IP, which affects the primary variable (prohibited medications)

Equivalence of ENZ215 to Prolia was also demonstrated in the secondary estimand analysis using principal stratum in the PP set, excluding patients experiencing ICEs:

Table 28. Percentage change in BMD at lumbar spine (L1-L4 region) measured by DXA from baseline to month 12 to assess equivalence of ENZ215 compared to Prolia - Secondary estimand analysis using principal stratum (PP set)

Treatment Group	n	Adjusted LS Mean (SE)	95% CI of adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference
ENZ215 (N = 230)	230	5.351 (0.3160)	4.7303; 5.9721	-0.190	-0.9149; 0.5342
Prolia® (N = 233)	233	5.542 (0.3117)	4.9291; 6.1541		

CI = Confidence Interval; N = The total number of patients in PP Set; n = Number of patients with non-missing data within the specific category.
BMD = Bone Mineral Density; DXA = Dual-energy X-ray absorptiometry; SE = Standard Error; LS Mean = Least Square Mean.
Analysis will be performed using an Analysis of Covariance (ANCOVA) model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates.
Patients who experience intercurrent events (ICEs) are excluded from the principal stratum analysis.
Equivalence will be established if 95% CIs of LS Mean difference lies between -1.45 and 1.45.

Supplementary analyses

The following revised version of the supplementary analysis was requested during the assessment. It targets an estimand based on the hypothetical strategy for all identified intercurrent events investigated using an MMRM approach.

Table 29. Percentage change in BMD at lumbar spine (L1-L4 region) measured by DXA from baseline to month 12 to assess equivalence of ENZ215 compared to Prolia - Primary estimand analysis with EMA suggested intercurrent events (ICEs) (ITT Set)*

Visit	Treatment Group	n	Adjusted LS Mean (SE)	95% CI of adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference	p-Value
Visit 7 (M6D180)	ENZ215 (N = 253)	216	3.655 (0.2625)	3.1390; 4.1708	-0.444	-1.0632; 0.1757	0.1598
	Prolia (N = 251)	217	4.099 (0.2611)	3.5854; 4.6118			
Visit 9 (M12D360)	ENZ215 (N = 253)	211	5.369 (0.2969)	4.7854; 5.9527	-0.319	-1.0486; 0.4105	0.3905
	Prolia (N = 251)	212	5.688 (0.2958)	5.1068; 6.2695			

CI = Confidence Interval; ITT = Intent-To-Treat Population Flag; N = The total number of patients in ITT Set; n = Number of patients with non-missing data within the specific category.
BMD = Bone mineral density; DXA = Dual energy X ray absorptiometry; SE= Standard Error; LS Mean = Least Square Mean.
*: The intercurrent events used in the analysis are delay in BMD assessment deviation by +/- 14 days at visit 7 and visit 9, disallowed medications according to Protocol section 6.11 (at any timepoint), receiving the second IP administration outside +/- 14 days of the scheduled date, treatment discontinuation at month 6 (ICE 3) and occurrence of vertebral fractures, lumbar spine surgery or other AEs with bone involvement in the lumbar area (ICE 4).
Analysis is performed with a mixed model with repeated measures with observed percentage change from baseline (%CfB) in lumbar spine BMD as the dependent variable and treatment, age strata, previous treatment with bisphosphonate, visit*treatment interaction and baseline BMD value as covariates.
Source: Responses #2, Appendix 7

Secondary Efficacy Endpoints

Percentage Change in BMD at Lumbar Spine Measured by DXA From Baseline to Month 6

Table 30. Percentage Change in BMD at Lumbar Spine (L1-L4 Region) Measured by DXA From Baseline to Month 6 - ANCOVA (ITT Set)

Treatment Group	n	Adjusted LS Mean (SE)	95% CI of adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference	p-value
ENZ215 (N = 253)	240	3.615 (0.2643)	3.0952; 4.1338	-0.389	-0.9937; 0.2150	0.2062
Prolia® (N = 251)	246	4.004 (0.2596)	3.4939; 4.5139			

Abbreviations: ANCOVA = analysis of covariance; BMD = bone mineral density; CI = confidence interval; DXA = dual-energy X-ray absorptiometry; ITT = intent-to-treat; LS Mean = least square mean; N = total number of participants in ITT Set; n = number of participants with non-missing data within the specific category; SE = standard error.
Analysis was performed using an ANCOVA model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates.
Source: Table 14.2.1.5.

Percentage Change in BMD at Total Hip and Femoral Neck Measured by DXA From Baseline to Month 6 and Month 12

Parameter: Total Hip

Table 31. Summary statistics for percentage change in BMD at total hip and femoral neck measured by DXA from baseline over time by treatment group (ITT set)

		ENZ215 (N=253)				Prolia® (N=251)			
Visit/(Day)	Statistics	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline
Baseline (D1)	n	253				251			
	Mean	0.7460				0.7600			
	SD	0.0878				0.0923			
	Minimum	0.547				0.552			
	Median	0.7450				0.7650			
	Maximum	0.978				1.064			
Visit 7 (M6D180)	n	240	240	240	240	244	244	244	244
	Mean	0.7469	0.7664	0.0195	2.693	0.7601	0.7800	0.0200	2.692
	SD	0.0880	0.0878	0.0215	2.9231	0.0920	0.0919	0.0179	2.4810
	Minimum	0.547	0.562	-0.068	-7.52	0.552	0.546	-0.046	-5.62
	Median	0.7450	0.7635	0.0190	2.635	0.7655	0.7830	0.0190	2.585
	Maximum	0.978	1.031	0.089	13.10	1.064	1.075	0.066	10.68
Visit 9 (M12D360)	n	231	231	231	231	235	235	235	235
	Mean	0.7475	0.7696	0.0221	3.026	0.7604	0.7851	0.0247	3.327
	SD	0.0884	0.0886	0.0200	2.7440	0.0921	0.0921	0.0189	2.5894
	Minimum	0.547	0.553	-0.044	-4.87	0.552	0.546	-0.038	-6.00
	Median	0.7470	0.7690	0.0220	2.960	0.7650	0.7900	0.0260	3.340
	Maximum	0.978	1.037	0.111	13.79	1.064	1.071	0.088	12.83

Parameter: Femoral Neck

		ENZ215 (N=253)				Prolia® (N=251)			
Visit/(Day)	Statistics	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline	Baseline	Post-baseline	Change from Baseline	Percentage Change from Baseline
Baseline (D1)	n	253				251			
	Mean	0.6705				0.6881			
	SD	0.1103				0.1040			
	Minimum	0.460				0.465			
	Median	0.6670				0.6740			
	Maximum	0.969				1.070			

Visit 7 (M6D180)	n	240	240	240	240	245	245	245	245
	Mean	0.6713	0.6837	0.0125	1.967	0.6889	0.7014	0.0124	1.861
	SD	0.1110	0.1109	0.0223	3.3099	0.1048	0.1054	0.0206	3.0835
	Minimum	0.460	0.475	-0.070	-8.02	0.465	0.448	-0.046	-8.68
	Median	0.6670	0.6780	0.0120	1.865	0.6740	0.6910	0.0120	1.840
	Maximum	0.969	1.013	0.079	10.28	1.070	1.059	0.078	10.58
Visit 9 (M12D360)	n	231	231	231	231	235	235	235	235
	Mean	0.6722	0.6868	0.0146	2.262	0.6890	0.7087	0.0198	2.938
	SD	0.1118	0.1132	0.0252	3.6223	0.1040	0.1060	0.0250	3.7590
	Minimum	0.460	0.473	-0.074	-9.85	0.465	0.434	-0.052	-8.83
	Median	0.6670	0.6810	0.0160	2.480	0.6750	0.7010	0.0200	2.820
	Maximum	0.969	1.030	0.126	15.71	1.070	1.063	0.127	20.00

ITT = Intent-To-Treat Population Flag; SD = Standard Deviation; N = The total number of patients in ITT Set; n = Number of patients with non-missing data within the specific category.
BMD = Bone Mineral Density; DXA = Dual-energy X-ray absorptiometry;
Source: Listing 16.2.6.1

Table 32. Percentage Change in BMD at Total Hip and Femoral Neck Measured by DXA From Baseline to Month 12 - ANCOVA (ITT Set)

Parameter	Treatment Group	n	Adjusted LS Mean (SE)	95% CI of Adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference	p-value
BMD at total hip	ENZ215 (N = 253)	231	2.534 (0.2044)	2.1321; 2.9357	-0.401	-0.8691; 0.0665	0.0926
	Prolia® (N = 251)	235	2.935 (0.2012)	2.5397; 3.3307			
BMD at femoral neck	ENZ215 (N = 253)	231	1.826 (0.2905)	1.2554; 2.3972	-0.763	-1.4285; -0.0970	0.0248
	Prolia® (N = 251)	235	2.589 (0.2866)	2.0258; 3.1523			

Abbreviations: ANCOVA = analysis of covariance; BMD = bone mineral density; CI = confidence interval; DXA = dual-energy X-ray absorptiometry; ITT = intent-to-treat; LS Mean = least square mean; N = total number of participants in ITT Set; n = number of participants with non-missing data within the specific category; SE = standard error.
Analysis was performed using an ANCOVA model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates.
Source: Table 14.2.1.9.

Table 33. Percentage change in BMD at total hip and femoral neck measured by DXA from baseline to month 12 - Analysis of covariance (ANCOVA) (PP set)

Parameter	Treatment Group	n	Adjusted LS Mean (SE)	95% CI of adjusted LS Mean	LS Mean Difference	95% CI of LS Mean Difference	p-value
BMD at total hip	ENZ215 (N = 230)	230	2.548 (0.2047)	2.1459; 2.9505	-0.374	-0.8436; 0.0953	0.1180
	Prolia® (N = 233)	233	2.922 (0.2016)	2.5262; 3.3185			
BMD at femoral neck	ENZ215 (N = 230)	230	1.823 (0.2917)	1.2498; 2.3964	-0.769	-1.4390; -0.0991	0.0246
	Prolia® (N = 233)	233	2.592 (0.2879)	2.0263; 3.1580			

CI = Confidence Interval; PP = Per-Protocol; SE = Standard Error; N = The total number of patients in PP Set; n = Number of patients with non-missing data within the specific category.
BMD = Bone Mineral Density; DXA = Dual-energy X-ray absorptiometry; LS Mean = Least Square Mean.
Analysis will be performed using an Analysis of Covariance (ANCOVA) model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates.
Source: Listing 16.2.6.1

The statistically significant difference between Prolia and ENZ215 (in favour of Prolia) for the percentage change from baseline to Month 12 in the BMD at femoral neck has been investigated with no evident cause being identified. There were no differences in PK, immunogenicity, potency or quality, batches used that could explain this result. Hence, this is considered as a chance imbalance arising from the higher variability for BMD at femoral neck and total hip compared to lumbar spine.

- **Ancillary analyses**

Comparison and Analysis of Results Across Studies

Efficacy data from Study No. ALK22/ENZ215-DEN2 can be compared to the pivotal study (FREEDOM study) performed with denosumab in postmenopausal women with osteoporosis. In the pivotal clinical study of Prolia (FREEDOM study) and its open-label extension study in patients with PMO for 10 years, significant mean percentage changes in BMD have been established at the lumbar spine, total hip, and femoral neck (Bone et al., 2017).

Table 34. Comparison Between Baseline Demographics of Study 0744-19 and FREEDOM Study

Parameters	FREEDOM (N=7808)		ALK22/ENZ215-DEN2 (N=504)	
	Placebo (N = 3906)	Denosumab (N = 3902)	Prolia (N = 253)	ENZ215 (N = 251)
Age (year), mean (SD)	72.3 (5.2)	72.3 (5.2)	66.1 (6.35)	66.3 (6.56)
Body mass index (kg/m^2), mean (SD)	26.0 (4.2)	26.0 (4.1)	25.48 (3.87)	25.26 (3.65)
Prevalent vertebral fracture, n (%)	915 (23%)	929 (24%)	40 (15.9%)	30 (11.9%)
Sources: Bolognese et al, 2013 and CSR Study ALK22/ENZ215-DEN2				

Table 35. Comparison of Week 52 (FREEDOM Study) and Month 12 (ALK22/ENZ215-DEN2) Efficacy Between Similar Design Studies in Postmenopausal Woman With Osteoporosis

		FREEDOM Study		**ALK22/ENZ215-DEN2**	
		Prolia (N = 3902)	**Placebo (N = 3906)**	**ITT set N=504**	
				Denosumab (N = 253)	Prolia (N = 251)
Percentage Change from Baseline in BMD	**Lumbar Spine**	5.5%	0.0%	5.775%	5.881%
	Total Hip	3.2%	-0.1%	3.026%	3.327%
	Femoral Neck	2.9%	0.0%	2.262%	2.938%
Sources: Bolognese et al., 2013; Prolia, USPI 2024, ALK22/ ENZ215-DEN2Table 14.2.1.1, 14.2.1.7					

In FREEDOM study, the largest placebo adjusted treatment effect size was observed in lumbar spine compared to total hip at Month 12. Effect size was 5.4 (5.4 [Prolia] vs 0.0 [Placebo]) for lumbar spine and 3.0 (3.2 [Prolia] vs 0.2 [Placebo]) for total hip (Bone et al. 2017). Similar results were reported in ALK22/ENZ215-DEN2. The effect size of ENZ215 for lumbar spine was 5.78 in ITT set, and for total hip was 3.026 in ITT set at Month 12.

- **Summary of main efficacy results**

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

Table 36. Summary of efficacy for trial ALK22/ENZ215-DEN2

Title: A Phase 3, Randomised, Double-blind, Parallel-group, Active-controlled Study to Compare the Efficacy, Safety, Pharmacodynamics, Pharmacokinetics and Immunogenicity of Enzene Denosumab (ENZ215) and Prolia in Postmenopausal Women with Osteoporosis.			
Study identifier	ALK22/ENZ215-DEN2 EudraCT Number 2021-004811-26		
Design	This was a Phase 3, randomised, double-blind, parallel-group, active-controlled study to compare the efficacy, safety, pharmacodynamics (PD), pharmacokinetics (PK), and immunogenicity of ENZ215 with Prolia in postmenopausal women with osteoporosis. The study was divided into three periods: Screening period (up to 35 days); double-blind treatment period of 12 months; and open-label, switch-over period of six months. All eligible participants were randomised in the double-blind treatment period in a 1:1 ratio to receive either ENZ215 or Prolia (60 mg) subcutaneously on Day 1 and Month 6. A PK sub-study was conducted in a subset of 120 participants with 60 participants in each arm. A subset of 120 participants randomised to Prolia arm were offered to enrol in the open-label, switch-over extension period.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	12 months (Double-blind treatment period) Not applicable 6 months (Open-label extension period)	
Hypothesis	Equivalence		
Treatments groups	ENZ215	ENZ215 60 mg on Day 1 and Month 6 for all participants and on Month 12 for participants in the open-label extension period 253 participants randomized and treated during the double-blind treatment period 60 participants entered the open-label extension period	
	Prolia	Prolia 60 mg on Day 1 and Month 6 for all participants and on Month 12 for participants in the open-label extension period 251 participants randomized and treated during the double-blind treatment period 60 participants entered the open-label extension period	
Endpoints and definitions	Co-Primary endpoints	Co-Primary Endpoint 1	Percentage change in bone mineral density (BMD) at lumbar spine (L1-L4 region) from baseline to Month 12
		Co-Primary Endpoint 2	Area under the effect curve (AUEC) of serum C-telopeptide of type 1 collagen (sCTX) over the initial six months (from Day 1 pre-dose to Month 6 pre-dose)

	Secondary endpoints	Secondary Endpoint 1	Percentage change in serum procollagen type 1 N-terminal propeptide (sP1NP) concentration from baseline to Month 1, Month 3 and Month 6
		Secondary Endpoint 2	Percentage change in BMD at lumbar spine from baseline to Month 6
		Secondary Endpoint 3	Percentage change in BMD at total hip and femoral neck from baseline to Month 6 and Month 12
Database lock	04 Sep 2024		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent-to-treat (ITT) set for Co-Primary Endpoint 1, Secondary Endpoint 2, and Secondary Endpoint 3. The ITT analysis set consisted of all randomised participants who received at least one dose of study intervention in the double-blind treatment period. PD set for Co-Primary Endpoint 2 and Secondary Endpoint 1. The PD set consisted of all participants in the safety set whose sCTX values were available in order to calculate PD parameter AUEC values for primary analysis and had no major protocol deviations which affected sCTX or sP1NP measurement. Timepoints analysed (depending on the endpoint): Month 1, Month 3, Month 6, and Month 12.		
Descriptive statistics and estimate variability	Treatment group	ENZ215	Prolia
	Number of participants in the ITT set	253	251
	Number of participants in the PD set	234	237
	Co-Primary Endpoint 1 (Adjusted Least Square [LS] Mean [standard error (SE)])	5.350 (0.3152)	5.533 (0.3108)
	95% confidence interval (CI) of Adjusted LS Mean	4.7306; 5.9695	4.9225; 6.1440
	Co-Primary Endpoint 2 (Geometric Mean)	347382.787	345858.423
	95% CI of Geometric Mean	340087.2488; 354834.8289	338640.7259; 353229.9557
	Secondary Endpoint 1 (Mean)	Month 1: -17.7485 Month 3: -75.7738 Month 6: -69.7567	Month 1: -18.3767 Month 3: -74.5419 Month 6: -69.1690
	Standard deviation	Month 1: 17.1910 Month 3: 15.4099 Month 6: 19.4086	Month 1: 23.4844 Month 3: 16.6630 Month 6: 22.8816

	Secondary Endpoint 2 (Adjusted LS Mean [SE])	3.615 (0.2643)	4.004 (0.2596)
	95% CI of Adjusted LS Mean	3.0952; 4.1338	3.4939; 4.5139
	Secondary Endpoint 3 (Adjusted LS Mean [SE])	Total Hip: 2.222 (0.2042) Femoral neck: 1.700 (0.2461)	Total hip: 2.327 (0.2005) Femoral neck: 1.688 (0.2420)
	95% CI of Adjusted LS Mean	Total hip: 1.8208; 2.6232 Femoral neck: 1.2162; 2.1834	Total hip: 1.9329; 2.7209 Femoral neck: 1.2120; 2.1632
Effect estimate per comparison	Co-Primary Endpoint 1	Comparison groups	ENZ215 and Prolia
		LS Mean Difference	-0.183
		95% CI of LS Mean Difference	-0.9044; 0.5380
		Analysis of covariance (ANCOVA)	Equivalence established as 95% CIs of LS Mean difference lied between -1.45 and 1.45
	Co-Primary Endpoint 2	Comparison groups	ENZ215 and Prolia
		Ratio of Geometric Means	1.004
		95% CI of Geometric Mean Ratio	0.975; 1.035
		ANCOVA	No accepted equivalence range, but 95% CI sufficiently close around unity to conclude on PD similarity
	Secondary Endpoint 1	Not applicable	
	Secondary Endpoint 2	Comparison groups	ENZ215 and Prolia
		LS Mean Difference	-0.389
		95% CI of LS Mean Difference	-0.9937; 0.2150
		ANCOVA	P-value=0.2062
	Secondary Endpoint 3	Comparison groups	ENZ215 and Prolia
		LS Mean Difference	Total hip: -0.105

			Femoral neck: 0.012
		95% CI of LS Mean Difference	Total hip: -0.5727; 0.3629 Femoral neck: -0.5520; 0.5765
		ANCOVA	Total hip: P-value=0.6598 Femoral neck: P-value=0.9660
Notes	Of the 504 randomised and treated participants, 469 participants overall (93.1%) completed the double-blind treatment period (233 participants [92.1%] in the ENZ215 group and 236 participants [94.0%] in the Prolia group) and 35 participants overall (6.9%) discontinued from the treatment and from the study during the double-blind treatment period (20 participants [7.9%] in the ENZ215 group and 15 participants [6.0%] in the Prolia group). The most frequent reasons for treatment discontinuation were withdrawal by participant (14 participants [5.5%] in the ENZ215 group and 10 participants [4.0%] in the Prolia group), followed by protocol violation (three participants [1.2%] in the ENZ215 group and no participants [0%] in the Prolia group), adverse event (one participant [0.4%] in the ENZ215 group and two participants [0.8%] in the Prolia group) and other (two participants [0.8%] in the ENZ215 group and one participant [0.4%] in the Prolia group). The reasons under the category other were "exclusion by Sponsor due to use of prohibited medication" for the two participants in the ENZ215 group and "more than 28 days elapsed from target date for Visit 7/Month 6" for the one participant in the Prolia group. Overall, 60 participants in the ENZ215 group and 60 participants in the Prolia group entered the open-label extension period. All 120 participants completed the study.		
Analysis description	**Pre-specified sensitivity analysis - Primary estimand analysis using multiple imputation (treatment policy estimand) in the ITT Set**		
Analysis population and time point description	ITT Set; Month 12		
Descriptive statistics and estimate variability	Treatment group	ENZ215	Prolia
	Co-Primary Endpoint 1 (Adjusted LS Mean [SE])	5.347 (0.3157)	5.542 (0.3112)
	95% CI of Adjusted LS Mean	4.7283; 5.9659	4.9324; 6.1524
Effect estimate per comparison	Co-Primary Endpoint 1	Comparison groups	ENZ215 and Prolia
		LS Mean Difference	-0.195
		95% CI of LS Mean Difference	-0.9167; 0.5259

		ANCOVA	Equivalence established as 95% CIs of LS Mean difference lied between -1.45 and 1.45.

2.5.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Study design: Overall, the design of the study ALK22/ENZ215-DEN2 is acceptable and has been discussed during EMA Scientific Advice procedures (EMA/CHMP/SAWP/531236/2020; EMEA/H/SA/4580/1/2020/III; EMA/SA/0000060480). Specific design aspects that were not followed or were not conducted as initially planned, will be discussed in the text below.

Study population: The main inclusion criteria were "postmenopausal women", "age between 55-80 years", "body weight between 50 and 90 kg. Another inclusion criterion was "absolute BMD consistent with T-scores of ≤ -2.5 and ≥ - 4.0 at the lumbar spine (L1-L4 region) as measured by dual-energy X ray absorptiometry (DXA) at screening". The inclusion of postmenopausal women with a T-score of ≥-4.0 and ≤-2.5 is in line with state of art definition of the WHO. In general, eligibility criteria are acceptable.

During the third protocol amendment (16 Feb 2023), which occurred while the study was already ongoing (recruitment start: 22 Oct 2021), multiple eligibility criteria were changed. Changes to the protocol during an ongoing study are seen very critical. The protocol amendments are listed below in section "Changes in the planned conduct of the study". With the third protocol amendment, the Applicant added an eligibility condition (patients should be excluded if cumulative use was > 3 years). Nevertheless, the Applicant instead stratified randomisation for earlier bisphosphonate use. This solution was recommended as alternative by CHMP during the SA procedures and is considered acceptable. Furthermore, some exclusion criteria/prohibited medication were added (e.g., romosozumab, antidepressants such as SSRIs, SNRIs, antipsychotics, romosozumab) or loosened (e.g., to allow intravaginal estrogen treatment). This can be accepted. In general, the list of prohibited medications is deemed rather extensive considering the study population. Overall, the inclusion and exclusion criteria are considered acceptable for recruitment of a population consisting of postmenopausal women with a diagnosis of osteoporosis.

In addition, it is agreed that the chosen study population (PMO patients) is appropriate to conduct a biosimilar study with denosumab, since PMO patients are a target patient population of the RMP. Furthermore, efficacy results can be extrapolated to all indications of Prolia due to the same mechanism of action for the three indications.

Randomisation and blinding: The CHMP recommended stratification with respect to age, weight, prior use of bisphosphonate, and geographic area. In the DEN2 study, participant allocation was stratified by age (≥ 55 to < 70 years and ≥ 70 to ≤ 85 years) and based on prior use of bisphosphonate. The average weight was similar between the treatment arms, i.e. weight can be described as balanced between treatment arms even though it was not included as stratification factor as recommended by the CHMP. The DEN2 study was only conducted in European study centres. This is endorsed and makes the CHMP's recommendation during the SA procedure to stratify for geographic region less relevant. In summary, the randomization procedure is considered acceptable.

The study employed a double-blind design until month 12, where patients and Investigators were blinded to study intervention. The process of blinding was adequately described and is considered acceptable.

Trial intervention: The chosen dose of 60 mg every 6 months (s.c.) is consistent with the posology recommendations of Prolia and is regarded adequate for the assessment of biosimilarity of the test and reference product. All enrolled subjects additionally received calcium (at least 1 g/day) and vitamin D (at least 400 IU/day) supplementation from screening until the end of the study, which is endorsed. This is in line with the clinical efficacy and safety studies for the initial marketing authorization of the reference product.

Upon request, the Applicant clarified that the manufacturing lot numbers/batch numbers for the study interventions dispensed in the Phase 3 DEN2 study were sourced from Enzene Drug Product (DP).

Concomitant therapy: Several medications were prohibited during the study. These included Xgeva, drugs used for treatment of osteoporosis, and drugs affecting bone metabolism. In general, this is endorsed. It should be noted that prohibited medications have a direct impact on the analysis of %CfB in LS-BMD at month 12 as this analysis takes the use of prohibited medication as intercurrent event ICE2 into account.

Study assessments: According to the protocol, the efficacy parameter BMD was assessed using the same DXA machine for all study procedures for a particular patient. The results were evaluated by a central assessor. This is regarded acceptable. Additionally, the Applicant provided a well-structured schedule of activities, which is endorsed.

Primary objectives, endpoints, estimands: The co-primary efficacy variable of "percentage change from the baseline in LS-BMD at Month 12" was based on DXA scans at the lumbar spine. The prespecified equivalence margins and corresponding confidence level were [1.45%, +1.45%] and 95%, which was agreed to during EMA scientific advice.

The primary estimand ('treatment policy estimand') seems to target an intention-to-treat analysis. Initially, the estimand only considered two types of intercurrent events: *significant BMD assessments delays for more than 35 days at visit 9* and *prohibited medication* and during assessment the following additional intercurrent events were identified: *treatment discontinuation, deviation in second IP administration, occurrence of vertebral fractures, lumbar spine surgery, or other adverse events involving the lumbar spine during the study*. Upon request, the Applicant clarified that a total of 26 subjects received the first but not the second dose, scheduled at Month 6 dose (ENZ215: 16 subjects, Prolia: 10 subjects). These discontinuations were primarily due to subject consent withdrawal, loss to follow-up, protocol deviations, or investigator decisions. Additionally, two adverse events were identified that could potentially impact lumbar spine BMD: a worsening of an L3 vertebral fracture in subject 60131031 (Prolia group), and an exacerbation of degenerative lumbar spine disease in subject 60231033 (ENZ215 group).

Moreover, for the primary estimand, the intercurrent event *prohibited medication* is described to have been targeted by a composite variable strategy, but it remains unclear how prohibited medication could have been taken to be a component of the change from baseline in BMD variable. For the primary analysis of the primary estimand, observations under prohibited medication were imputed under the assumption of MAR in the Prolia arm and under an assumption of MNAR in the ENZ215 arm, which does not seem to reflect the intended composite variable strategy.

For the assessment, a modified version of the proposed primary estimand taking into account the additional intercurrent events '*treatment discontinuation'* and '*interventions concerning the area relevant for DXA measurement'* and applying the treatment policy strategy to all intercurrent events will be considered. Applying a principal stratum strategy to all intercurrent events, as proposed for the secondary estimand, is considered difficult to interpret. Instead, the analysis targeting an estimand based on the hypothetical

strategy which was provided without specifying the details on this estimand, will be considered as complementary information, providing a per-protocol perspective.

In addition to the BMD analysis, the Applicant included the AUEC of sCTX (a PD marker) over the initial six months (from Day 1 pre-dose to Month 6 pre-dose) as co-primary endpoint, as recommended in the scientific advice. This is endorsed. During the third protocol amendment (16 Feb 2023), the confidence level to be employed in the assessment of equivalence for the co-primary PD analysis was changed from 95% to 90%CI. This change to the 90%CI is not endorsed. Nevertheless, since the Applicant also provided results of the 95%CI, the 95%CI is assessed, and no concern is raised. Moreover, the proposed acceptance range of 80-125% for the PD endpoint $AUEC_{0\text{-}6months}$ is based on margins used for conventional bioequivalence analyses without further justification. The acceptance range of 80–125% is not appropriate per se, but as the provided results are considered clear enough to support equivalence, this issue is not further pursued.

Secondary objectives, endpoints: The secondary objectives included PK, PD, efficacy, safety and immunogenicity aspects of ENZ215 and EU Prolia. Overall, the secondary objectives of the study are endorsed, as already discussed during scientific advice.

Statistical methods: The ITT analysis set, which was the analysis set to be used for the primary analysis, consisted of all randomised participants who received at least one dose of study intervention in the double-blind treatment period. This is acceptable. The primary analysis of the primary estimand ('treatment policy estimand') was based on an analysis of covariance (ANCOVA) model including treatment, age strata, previous treatment with bisphosphonate and baseline BMD value as covariates. Missing data without experiencing intercurrent event 2 (ICE2) were planned to be assumed to be MCAR and were not to be imputed. BMD values at 12 months which were assessed with more than 35 days of delay (ICE1) were to be included in the analysis as observed in line with the intention-to-treat principle. For patients who used prohibited medication (ICE2), data in the Prolia arm was imputed under the assumption of missing at random, whereas data in the ENZ215 arm was imputed assuming missing not at random, by first imputing under MAR and then shifting by -1.45%.

As discussed in the estimand section, the definition of the primary estimand is considered insufficient. Consequently, the question to which extent the primary analysis reflects the primary estimand is of little value. Instead, it needs to be discussed whether the treatment effect estimated by the primary analysis is relevant for the benefit-risk assessment. Using the observed BMD values for patients with BMD assessment delays (intercurrent event 1) is considered reasonable and in line with the intention-to-treat principle. However, applying different imputation strategies depending on the treatment arms in patients with prohibited medication is not considered to target an easily describable treatment effect, even if it might be relevant as part of a tipping point analysis. The same concern holds for all sensitivity analyses provided for the primary estimand. Thus, upon request the Applicant provided an analysis targeting an estimand similar to the primary estimand defined in the SAP but where treatment discontinuation and lumbar spine related adverse events are considered as additional intercurrent event and the treatment policy strategy is applied to all intercurrent events. This analysis used observed data (if available) and imputed missing data after intercurrent events as values drawn from a normal distribution centered around 0. While this simplistic imputation strategy might be agreed to generate values in line with the treatment policy strategy for the intercurrent event treatment discontinuation (even though the variability of the imputed values is considered low), the imputation strategy is not considered suitable to impute values after prohibited medication and delay in BMD assessment. However, as treatment discontinuation is understood to be the intercurrent event related to the largest amount of missing values and as the resulting confidence interval is small compared to the equivalence range, this issue is not pursued further. Missing values not related to intercurrent events were imputed under the assumption of MAR, similarly as performed for some of the sensitivity analyses.

Additionally, the Applicant investigated an estimand based on a hypothetical strategy for the two identified intercurrent events BMD assessment delay and prohibited medication using an MMRM approach excluding all data points after intercurrent events. Missing data was not to be imputed, which is considered acceptable in combination with the MMRM. Upon request, a revised analysis was provided taking into account *treatment discontinuation*, *deviation in second IP administration* and *lumbar spine-related adverse events* as intercurrent events and using a stricter definition of the intercurrent events *delay in BMD assessment* and *use of prohibited medications*. Generally, the MMRM analysis is endorsed.

Changes in planned study conduct: Overall, the protocol amendments are regarded acceptable.

Baseline characteristics:

Demography: Although the CHMP generally recommended to exclude all patients with previous exposure of i.v. bisphosphonates because of very long-term residual effects, the Applicant was recommended to alternatively stratify by prior bisphosphonate use and analyze any potential imbalances caused by prior bisphosphonate exposures in stratified sensitivity analyses. This alternative recommendation was followed by the Applicant which is acceptable. The majority of the subjects, approx. 80%, were bisphosphonate naïve. Heavy smokers, smoking 20 or more cigarettes per day, were excluded. Overall, demographic baseline characteristics appear balanced between groups in the double-blind and open-label periods.

Baseline Disease Characteristics: A total of 228 participants (45.2%) reported a history of fracture. There was a slightly higher rate of previous fractures in the ENZ215 group (ENZ215: 118 participants [46.6%]; Prolia: 110 participants [43.8%]), which is a risk factor for subsequent fractures even after adjustment for BMD. The most frequently reported previous fractures reported by patients were thoracic vertebral fracture (ENZ215: 21 participants [8.3%]; Prolia: 29 participants [11.6%]), causing a slight excess in previous fractures in the Prolia group compared to the ENZ215 group. At screening, a similar extent of vertebral fractures could be determined via X-ray (ENZ215: 11.9%; Prolia: 15.9%). Furthermore, wrist fractures were more common in the ENZ215 group (10.3%) compared with the Prolia group (7.2%). Nevertheless, this small imbalance is not thought to have a considerable influence on efficacy results, overall. Fractures occurring *during* the study are discussed in the safety section.

Medical History and Concurrent Illnesses: Most of the participants reported medical history (ENZ215: 95.7%; Prolia: 93.2%), mainly due to the Musculoskeletal and Connective Tissue Disorders (ENZ215: 58.5%; Prolia: 57.8%) and the Vascular Disorders SOC (EN215: 51.0%; Prolia: 46.2%). Considering the overall study population (PMO patients ≥55 yo), the medical history appears sufficiently balanced between treatment groups.

Prior and Concomitant Therapy: More patients in the ENZ215 group had prior medication (ENZ215: 81.4%; Prolia: 76.5%) and concomitant medication (double-blind period: ENZ215: 59.7%, Prolia: 52.2%; open-label period: ENZ215: 36.7%; Prolia: 28.3%) compared to the Prolia group. 17.3% of patients (ENZ215: 17.8%; Prolia: 16.7%) had used "drugs affecting bone structure and mineralization" prior to study entry which had to be discontinued per study protocol. During the double-blind period, major protocol deviations related to disallowed drugs affecting bone mineralization were reported in slightly more patients of the ENZ215 group (ENZ215: 2.4%; Prolia: 1.2%). All these subjects were excluded from the PP set which is endorsed. Upon request, the Applicant provided an updated summary table and a listing of protocol deviations concerning the DEN2 study. Overall, the population appears sufficiently balanced between arms and sensitive to assess biosimilarity.

Efficacy data and additional analyses

At baseline (D1), the mean (SD) BMD was comparable between the ENZ215 and the Prolia group. The number of patients with *intercurrent events* is considered low, but paradoxically (as discussed above), *protocol deviations* occurred very frequently, and *treatment discontinuation* were requested to be considered as additional intercurrent event. There were 12 patients (2.4%, 6 patients in ENZ215 arm and 6 patients in Prolia arm) with intercurrent event 1 (BMD assessment delays for more than 35 days) and 9 patients (1.8%, 6 patients in ENZ215 arm and 3 patients in Prolia arm) with intercurrent event 2 (prohibited medication).

The primary efficacy analysis gave an estimated difference in percentage change from baseline to month 12 of **lumbar spine BMD** between the ENZ215 and the EU-Prolia group of -0.183% (95% CI: -0.904%, 0.538%). Thus, the 95% CI is within the equivalence range of [-1.45%, 1.45%], i.e., the pre-specified biosimilarity criterion is fulfilled for the co-primary efficacy endpoint. The requested revised analysis (taking into account treatment discontinuation and lumbar spine-related adverse events as additional intercurrent events and implementing the treatment policy strategy for all four intercurrent events) resulted in an estimated treatment difference of -0.182 [95%CI: -0.896; 0.532]) which is in line with the claim of biosimilarity. Sensitivity analyses on the mITT set, on the PP set and using multiple imputation for missing data on the ITT set gave similar results as the original analysis on the ITT set.

Upon request, the Applicant provided a sensitivity efficacy analysis using an adapted MMRM model targeting a hypothetical estimand for the primary endpoint assuming there were no BMD assessment delays, no use of prohibited medication, no treatment discontinuations, no deviation in administration of second dose of IP and no lumbar spine-related adverse events. The outcome supports the robustness of the efficacy results and indicates that the protocol deviations observed in the DEN2 study did not compromise the conclusion of biosimilarity between ENZ215 and Prolia.

Subgroup analyses were neither planned nor performed. In this respect, subgroup analyses according to ADA and nAb status would have been of interest. However, considering that ADA development occurred in close to all patients whereas in contrast rate of nAb was very low, these analyses would not have been informative, therefore no issue is raised. See below assessment of clinical safety.

The difference in percentage change from baseline to month 6 in lumbar spine BMD between the ENZ215 and the EU-Prolia group was estimated to be -0.389% (95% CI: -0.994; 0.215)] using an ANCOVA model on the ITT set. Assessment of BMD at the lumbar spine was not part of the open-label extension period. While results for additional 6 months could have provided support for month 12, this is not a requirement and thus acceptable.

In addition to the lumbar spine BMD, the percent change from baseline in **BMD** was also measured in **total hip** and **femoral neck**. At Month 6, %CfB values between ENZ215 and Prolia treatment groups were comparable at total hip and femoral neck. However, at Month 12, %CfB in BMD at the femoral neck favored Prolia over ENZ215. While the %CfB in the Prolia group was of a similar magnitude to the %CfB reported in the RMP's pivotal trial ("FREEDOM" study), the %CfB was lower in the ENZ215 group. In face of the nominally statistically significant results (p=0.02, two-sided) for femoral neck at month 12 in the PP set, the Applicant performed investigations but could not identify any evident cause. According to the Applicant, there were no differences in PK, immunogenicity, potency or quality, batches used that could explain this result. Hence, the Applicant considered the difference as a chance imbalance arising from the higher variability for BMD at femoral neck and total hip compared to lumbar spine. This explanation can only be partially followed since BMD variability does not appear to be drastically different at the femoral neck compared to, e.g., the lumbar spine (Fig. 1 in Bolognese et al., 2013; DOI: 10.1016/j.jocd.2012.02.006). Nevertheless, the Applicant's explanation can be accepted in light of the results for other endpoints and supported by fracture rate

incidence. In addition to the Month 12 results at the femoral neck, a similar - though non-significant - trend favouring Prolia was observed for %CfB in total hip BMD (p = 0.09, two-sided).

Fractures were reported as TEAE only. A total of 24 fractures at various locations were reported during the 12 months study duration, 16 fractures in 10 patients in the ENZ215 group (4.0%) and 8 fractures in 7 patients in the Prolia group (2.8%). Two fractures in 2 patients in the Prolia group (3.3%) occurred during the open-label extension period. Thus, no clear imbalance in fractures was observed (please also refer to the Safety section).

The Applicant provided a **comparison** of efficacy data from pivotal study ALK22/ENZ215-DEN2 with the pivotal Prolia study (FREEDOM study) performed with denosumab in postmenopausal women with osteoporosis. Even though there were moderate differences between baseline characteristics of DEN2 and FREEDOM studies, the efficacy endpoint "%CfB in BMD after ~1 year treatment" was comparable between studies, measured at the lumbar spine, total hip, and femoral neck. It is reassuring that study results appear robust between products.

2.5.7. Conclusions on the clinical efficacy

The biosimilarity of ENZ215 and EU-Prolia in terms of efficacy was demonstrated in osteoporosis patients. The percentage change in BMD at Lumbar Spine (L1-L4 Region) measured by DXA from baseline to Month 12 was the co-primary efficacy endpoint in this study. Baseline characteristics were balanced between groups. The statistical analysis on the treatment policy estimand revealed that the LS-mean difference between the ENZ215 and the EU-Prolia group was -0.183% with the corresponding 95% CI being [-0.9044%, 0.5380%]. Thus, the 95% CI was within the pre-specified and accepted equivalence range of [-1.45%, 1.45%] and the co-primary efficacy endpoint was met. The primary analysis was further supported by analyses on an estimand based on the hypothetical strategy, sensitivity analyses, and secondary efficacy endpoints. A main uncertainty is the particularly high number of subjects with protocol deviations in the Phase 3 study (68.8% in the double-blind 12-months period and 40.0% in the 6-months extension period). Nevertheless, overall the results are considered robust and support the conclusion of efficacy biosimilarity between Osqay and EU-Prolia.

2.5.8. Clinical safety

Comparability of safety and immunogenicity of ENZ215 with the reference products Prolia was investigated in the following studies:

- ALK22/ENZ215-DEN1 was a Phase 1, randomized, double-blind, three-arm, parallel-group, single-dose study to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity of Denosumab (ENZ215, EU-sourced Prolia, and US-sourced Prolia) in healthy adult male volunteers.
- ALK22/ENZ215-DEN2 was a Phase 3, randomized, double-blind, parallel-group, active-controlled study to compare the efficacy, safety, PD, PK and immunogenicity of ENZ215 with EU-Prolia in postmenopausal women with osteoporosis (PMO).

Study ALK22/ENZ215-DEN1:

Adverse events and SAEs as and when occurred, were properly recorded, evaluated, managed, and reported from signing informed consent till end of Study Assessment visit.

Safety was evaluated throughout the study based on complete physical examination, including signs and symptoms of COVID-19, AE monitoring, vital signs (blood pressure, pulse rate, respiratory rate, and body temperature), 12-lead ECG, ADA and NAb assessment and laboratory investigations. Any clinically significant abnormalities including those present prior to the start of study medication were recorded as medical history. Adverse events persisting at the end of the study were followed up by the Investigator until resolution or until a clinically stable endpoint is reached.

All AEs were summarized using appropriate medical coding dictionary.

Safety and Immunogenicity Endpoints:

1. Number of subjects who developed denosumab NAb and ADAs (Day 1, 28, 90, 180, and 270).
2. Incidence of adverse events (AEs).
3. Clinically significant changes in physical examination findings, safety laboratory analyses (serum chemistry, hematology, and urinalysis), vital signs, and 12-lead ECG.

According to protocol Version 4.0, Dated 03/Oct/2022:

Severity of event

Adverse events will be assessed and graded to characterize the severity of the Adverse Event as per the latest version of CTCAE (Common Terminology Criteria for Adverse events). The severity of mild, moderate, severe, and life-threatening, death related to the AE corresponding respectively to Grades 1 –5, will be used.

For evaluating severity, following classification will be used to quantify intensity:

1: *Mild*: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

2: *Moderate*: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.)

3: *Severe or medically significant but not immediately life threatening*: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care Activities of Daily Living (refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden)

4: *Life threatening consequences*: urgent intervention indicated

5: *Death*: Death related to AE

Relationship to investigational products

The clinician's assessment of an AE's relationship to study medication is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AE's must have their relationship to investigational product assessed using the terms Related or Unrelated. In a clinical trial, the study product must always be suspect. This assessment will be based on following criteria:

- Temporal relationship
- Pharmacological plausibility

- Rechallenge and Dechallenge information
- Other confounding factors like underlying concurrent conditions, co-suspects, concomitant medications etc.

Study ALK22/ENZ215-DEN2:

Safety and Immunogenicity Endpoints:

- ADAs incidence at baseline (Day 1) and Months 1, 3, 6, 9 and 12 and during open-label switch-over period, i.e. Months 15 and 18
- Treatment-emergent serious and non-serious adverse events (TEAEs) during main treatment period and open-label switch-over period
- Alteration in clinical laboratory parameters during main treatment period and open-label switch-over period

According to protocol Version 3.0, Approval Date:16 February 2023:

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:

Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that causes sufficient discomfort to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category used for rating the intensity of an event; and both AE and SAE can be assessed as severe. An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. The Investigator will use clinical judgment to determine the relationship.

A *reasonable* possibility of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.

The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in their assessment.

For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report in the electronic data collection tool. However, it is very important that the

Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the electronic data collection tool.

The Investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

The following "binary" decision choice will be used by the Investigator to describe the initial causality assessment:

• Related: Reasonable possibility of a relatedness

• Not related: No reasonable possibility of relatedness.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

2.5.8.1. Patient exposure

Table 37. Patient exposure

	Patients enrolled	Patients exposed*	Patients exposed to the proposed dose range	Patients with long term** safety data
Blinded studies (placebo-controlled)	NA	NA	NA	NA
Blinded studies (active -controlled)	DEN1: 207	DEN1: 69	DEN1: 69	DEN1: 69
	DEN2: 504	DEN2: 253	DEN2: 253	DEN2: 253 + 60#
Open studies	NA	NA	NA	NA
Post marketing	NA	NA	NA	NA
Compassionate use	NA	NA	NA	NA

* Received at least 1 dose of active treatment
** In general this refers to 6 months and 12 months continuous exposure data, or intermittent exposure.

Patients from Prolia arm in double blind phase enrolled in ENZ215 arm in Open label extension phase

Study ALK22/ENZ215-DEN1:

All safety analyses were carried out using the safety analysis set, which was defined as all subjects who received a single-dose of the study drug. Subjects were classified according to treatment received.

Of the 344 subjects screened for the study, 207 were randomized (68 subjects to ENZ215 group, 69 to EU-sourced Prolia group, and 70 to US-sourced Prolia group). (Note: 69 subjects each were to be randomized to the 3 treatment groups. A trivial imbalance was observed in the number of subjects randomized to each treatment arm due to paper randomization being used to manage randomization in a multi-site trial. This situation of imbalance was a known and accepted risk, as this does not present any issue from a regulatory perspective).

All randomized subjects received study treatment except 1 (in US-sourced Prolia group) who had withdrawn consent after randomization. The majority of screen failures were due to subjects not fulfilling the eligibility criteria. The most frequent reason for screen failure was subjects not meeting inclusion criterion number 3

(ie, BMI criteria), inclusion criterion number 9 (ie, willing and able to comply with the protocol requirements) and meeting exclusion criterion number 20 (ie, any reason/condition which would preclude subject's participation in the study as per the Investigator's opinion or warnings and contraindications in the prescribing information of Prolia).

A total of 200 (96.6%) subjects completed the study, with a similar proportion of subjects in all 3 treatment groups. Overall, 7 (3.4%) subjects prematurely discontinued from the study. The reason for study discontinuation were lost to follow-up and missing visits (1 subject each) in ENZ215 group; lost to follow-up (1 subject) and withdrawal by subject due to personal reason (2 subjects) in US-sourced Prolia group; lost to follow-up (2 subjects) in EU-sourced Prolia group.

Study ALK22/ENZ215-DEN2:

The safety set included all randomised participants who received at least one dose of study intervention. In the safety set, treatment was assigned based on the actual treatment that participants received.

Double-blind treatment period:

Of the total 504 randomised and treated participants, the majority of the participants (94.8%) received two injections of study treatment: 237 participants (93.7%) in the ENZ215 group and 241 participants (96.0%) in the Prolia group. The mean (SD) exposure duration and study duration were similar between the two treatment groups: 351.4 (44.70) days and 350.1 (54.22) days, respectively, in the ENZ215 group and 356.2 (36.18) days and 353.6 [48.78] days, respectively, in the Prolia group. The mean (SD) total dose of calcium during the double-blind treatment period was similar between the two treatment groups (357.02 [70.786] g in the ENZ215 group and 364.47 [69.416] g in the Prolia group), with a mean daily intake per participant of 1.05 g in both treatment groups and a mean exposure of 343.0 days in the ENZ215 group and 348.3 days in the Prolia group. The mean (SD) total dose of vitamin D during the double-blind treatment period was similar between the two treatment groups (735298.51 [375097.869] IU in the ENZ215 group and 753055.68 [391445.986] IU in the Prolia group), with a mean daily intake per participant of 1575.43 IU in the ENZ215 group and 1599.00 IU in the Prolia group and a mean exposure of 342.4 days in the ENZ215 group and 349.0 days in the Prolia group.

Open-label extension period:

A subset of 120 participants randomised to Prolia arm and who completed 12 months of the double-blind treatment period without any significant safety concerns per the Investigator's discretion were offered to enroll in the open-label, switch-over extension period. The purpose of this open-label extension period of the study was to assess the impact on immunogenicity and safety of switching participants from Prolia to ENZ215. After re-consenting for the open-label, switch-over study, the participants were re-randomised, without any stratification, in a 1:1 ratio to receive either ENZ215 or Prolia (60 mg) SC at Month 12. These participants completed the study at Month 18.

All 120 participants (100.0%) who entered the open-label extension period received one injection of study treatment during the open-label extension period. The mean (SD) exposure duration was 181.0 (NC) days in both treatment groups. The mean (SD) study duration was similar between the treatment groups, 179.3 (7.18) days in the ENZ215 group and 181.1 (9.46) days in the Prolia group. The mean (SD) total dose of calcium during the open-label extension period was similar between the two treatment groups (182.38 [21.309] g in the ENZ215 group and 186.07 [21.815] g in the Prolia group), with a mean daily intake per participant of 1.04 g and 1.06 g in the ENZ215 group and Prolia group, respectively. The mean exposure duration was 176.4 days in the ENZ215 group and 177.3 days in the Prolia group. The mean (SD) total dose of vitamin D during the open-label extension period was similar between the two treatment groups

(378003.21 [209006.016] IU in the ENZ215 group and 398065.43 [216555.421] IU in the Prolia group), with a mean daily intake per participant of 1514.48 IU in the ENZ215 group and 1620.44 IU in the Prolia group and mean exposure duration of 177.8 days in the ENZ215 and 177.3 days in the Prolia group.

Table 38. Summary of Study Treatment Administration (Safety Set)

Treatment Group	ENZ215	Prolia®	Overall
Double-blind treatment period	(N = 253)	(N = 251)	(N = 504)
Number (%) of participants with injections			
1	16 (6.3)	10 (4.0)	26 (5.2)
2	237 (93.7)	241 (96.0)	478 (94.8)
Exposure Duration (days)			
n	253	251	504
Mean	351.4	356.2	353.8
SD	44.70	36.18	40.71
Minimum	181	181	181
Median	362.0	362.0	362.0
Maximum	393	393	393
Study Duration (days)			
n	253	251	504
Mean	350.1	353.6	351.8
SD	54.22	48.78	51.57
Minimum	15	29	15
Median	363.0	363.0	363.0
Maximum	415	407	415
Treatment Compliance (%)			
n	253	251	504
Mean	98.2	99.0	98.6
SD	9.28	7.00	8.22
Minimum	50	50	50
Median	100.0	100.0	100.0
Maximum	100	100	100
Open-label extension period	**(N = 60)**	**(N = 60)**	**(N = 120)**
Number (%) of participants with injections			
1	60 (100)	60 (100)	120 (100)
Exposure Duration (days)			
n	60	60	120
Mean	181.0	181.0	181.0
SD	NC	NC	NC
Minimum	181	181	181
Median	181.0	181.0	181.0
Maximum	181	181	181
Study Duration (days)			
n	60	60	120
Mean	179.3	181.1	180.2
SD	7.18	9.46	8.41
Minimum	162	164	162
Median	179.0	181.0	180.0
Maximum	197	228	228
Treatment Compliance (%)			
n	60	60	120
Mean	100.0	100.0	100.0
SD	NC	NC	NC
Minimum	100	100	100
Median	100.0	100.0	100.0
Maximum	100	100	100

Abbreviations: N = total number of participants in safety set; NC = not calculable; n = number of participants with non-missing data within the specific category; SD = standard deviation.
Exposure Duration (days) = [Date of last injection + 180 (days)] - [Date of 1st injection] + 1.
Study duration (days) = (Date of termination/completion/cut off - Date of 1st injection) + 1.
Compliance (%) = [(Number of Injections Administered)/(Number of Injections supposed to be administered)]*100.
Source: Table 14.1.8.1.

2.5.8.2. Adverse events

2.5.8.2.1. Healthy male population (Study ALK22/ENZ215-DEN1)

This section focuses primarily on treatment-emergent AEs (TEAEs), ie, AEs that started or worsened in severity on or after the administration of study treatment. All causality assessments mentioned in subsequent sections were performed by the Investigator. All AEs were classified according to the Medical Dictionary for Regulatory Activities (MedDRA), Version 25.0 for subjects of site 01 and from MedDRA dictionary, Version 26.1 for subjects of site 02 and 03.

Overall, 99 (48.1%) subjects experienced at least 1 TEAE, where the observed difference in incidence was slightly higher in EU-sourced Prolia group (39 [56.5%] subjects) compared with US-sourced Prolia (32 [46.4%] subjects) and ENZ215 groups (28 [41.2%] subjects).

The majority of the subjects reported either TEAEs of mild (83 [40.3%] subjects) or moderate (50 [24.3%] subjects) intensity while severe TEAEs were reported in 2 (1.0%) subjects.

- The incidence of mild TEAEs was slightly higher in EU-sourced Prolia group (33 [47.8%] subjects) compared with ENZ215 (25 [36.8%] subjects) and US-sourced Prolia groups (25 [36.2%] subjects).
- The moderate TEAEs had a lower incidence in ENZ215 group (12 [17.6%] subjects) compared with US-sourced Prolia (18 [26.1%] subjects) and EU-sourced Prolia groups (20 [29.0%] subjects).
- Severe TEAEs were reported only in ENZ215 (1 [1.5%] subject) and EU-sourced Prolia groups (1 [1.4%] subject).

Overall, 15 (7.3%) subjects experienced at least 1 TEAE considered as related to the study treatment, where the incidence was similar in all treatment groups.

Table 39. Overall Summary of All Treatment-emergent Adverse Events (Safety Analysis Set)

	ENZ215 (N=68)		US-Prolia® (N=69)		EU-Prolia® (N=69)		Total (N=206)	
	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n
TEAEs	28(41.2)	71	32(46.4)	70	39(56.5)	78	99(48.1)	219
Related TEAEs	5(7.4)	5	4(5.8)	4	6(8.7)	8	15(7.3)	17
Serious TEAEs	0	0	0	0	1(1.4)	1	1(0.5)	1
TEAEs leading to treatment discontinuation	0	0	0	0	0	0	0	0
TEAEs leading to death	0	0	0	0	0	0	0	0
TEAEs by severity	0	0	0	0	0	0	0	0
Mild	25(36.8)	42	25(36.2)	43	33(47.8)	50	83(40.3)	135
Moderate	12(17.6)	28	18(26.1)	27	20(29.0)	27	50(24.3)	82
Severe	1(1.5)	1	0	0	1(1.4)	1	2(1.0)	2
Life Threatening	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0

Abbreviation: EU = European Union; TEAE = treatment-emergent adverse event; US = United States.
Source: Table 14.3.1.1.

The incidence of TEAEs by SOC was similar across all the 3 treatment groups except infections and infestations and injury, poisoning and procedural complications. The observed difference in incidence of these SOCs was slightly higher in EU-sourced Prolia group compared with US-sourced Prolia and ENZ215 groups. The incidence of TEAEs by PT was similar across all the 3 treatment groups except nasopharyngitis and upper respiratory tract infections. The observed difference in incidence was slightly higher in US-sourced Prolia group compared with EU-sourced Prolia and ENZ215 groups and upper respiratory tract infection, where the observed difference in incidence was slightly higher in EU-sourced Prolia compared with ENZ215 and US-sourced Prolia groups.

The most commonly reported TEAEs by SOC were infections and infestations (13 [19.1%] subjects in ENZ215, 17 [24.6%] subjects in US-sourced Prolia, and 20 [29.0%] subjects in EU-sourced Prolia groups) and nervous system disorders (11 [16.2%] subjects in ENZ215, 14 [20.3%] subjects in US-sourced Prolia, and 12 [17.4%] subjects in EU-sourced Prolia groups).

The most commonly reported TEAEs by PT were nasopharyngitis (6 [8.8%] subjects in ENZ215, 13 [18.8%] subjects in US-sourced Prolia, and 11 [15.9%] subjects in EU-sourced Prolia groups) and headache (9 [13.2%] subjects in ENZ215, 14 [20.3%] subjects in US-sourced Prolia, and 9 [13.0%] subjects in EU-sourced Prolia groups).

Table 40. Summary of TEAEs by System organ Class and Preferred Term Occurring in >2% Subjects by Preferred Term in any of the Treatment Group (Safety Analysis set)

	ENZ215 (N=68)		US-Prolia® (N=69)		EU-Prolia® (N=69)		Total (N=206)	
System Organ Class/ Preferred Term	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n
Number of subjects with TEAEs	28 (41.2)	71	32 (46.4)	70	39 (56.5)	78	99 (48.1)	219
Gastrointestinal disorders	6 (8.8)	8	6 (8.7)	6	7 (10.1)	7	19 (9.2)	21
Aphthous ulcer	2 (2.9)	2	2 (2.9)	2	1 (1.4)	1	5 (2.4)	5
Toothache	0	0	1 (1.4)	1	3 (4.3)	3	4 (1.9)	4
Infections and infestations	13 (19.1)	16	17 (24.6)	21	20 (29.0)	28	50 (24.3)	65
Gastrointestinal infection	0	0	2 (2.9)	2	1 (1.4)	1	3 (1.5)	3
Gastrointestinal viral infection	0	0	2 (2.9)	2	0	0	2 (1.0)	2
Influenza	2 (2.9)	2	0	0	0	0	2 (1.0)	2
Nasopharyngitis	6 (8.8)	6	13 (18.8)	14	11 (15.9)	13	30 (14.6)	33
Rhinitis	0	0	1 (1.4)	1	2 (2.9)	2	3 (1.5)	3
Upper respiratory tract infection	3 (4.4)	3	1 (1.4)	1	7 (10.1)	7	11 (5.3)	11
Injury, poisoning and procedural complications	3 (4.4)	4	0	0	7 (10.1)	8	10 (4.9)	12
Skin abrasion	0	0	0	0	3 (4.3)	3	3 (1.5)	3
Investigations	2 (2.9)	2	4 (5.8)	4	6 (8.7)	6	12 (5.8)	12
SARS-CoV-2 test positive	2 (2.9)	2	1 (1.4)	1	6 (8.7)	6	9 (4.4)	9
Metabolism and nutrition disorders	3 (4.4)	3	2 (2.9)	2	3 (4.3)	3	8 (3.9)	8
Hypocalcaemia	3 (4.4)	3	2 (2.9)	2	3 (4.3)	3	8 (3.9)	8
Musculoskeletal and connective tissue disorders	3 (4.4)	3	3 (4.3)	4	6 (8.7)	7	12 (5.8)	14
Arthralgia	1 (1.5)	1	0	0	4 (5.8)	4	5 (2.4)	5
Back pain	2 (2.9)	2	1 (1.4)	1	0	0	3 (1.5)	3
Nervous system disorders	11 (16.2)	23	14 (20.3)	22	12 (17.4)	14	37 (18.0)	59
Dizziness	0	0	1 (1.4)	1	2 (2.9)	2	3 (1.5)	3
Headache	9 (13.2)	18	14 (20.3)	20	9 (13.0)	11	32 (15.5)	49
Migraine	2 (2.9)	3	0	0	0	0	2 (1.0)	3
Respiratory, thoracic and mediastinal disorders	5 (7.4)	5	5 (7.2)	5	1 (1.4)	1	11 (5.3)	11
Oropharyngeal pain	2 (2.9)	2	0	0	1 (1.4)	1	3 (1.5)	3
Rhinorrhoea	2 (2.9)	2	2 (2.9)	2	0	0	4 (1.9)	4
Skin and subcutaneous tissue disorders	3 (4.4)	3	1 (1.4)	1	0	0	4 (1.9)	4
Rash	3 (4.4)	3	0	0	0	0	3 (1.5)	3

Abbreviations: EU = European Union; MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event; US = United States.
Note(s): System organ class and preferred term are from the MedDRA dictionary, Version 25.0 for subjects of site 01 and from MedDRA dictionary, Version 26.1 for subjects of site 02 and 03.
The numbers of subjects within each column cannot be added because a subject may have had more than 1 adverse event.
A subject experiencing multiple occurrences of an adverse event was counted, at most, once per system organ class and preferred term.
Source: Table 14.3.1.2.

2.5.8.2.2. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

Double-blind treatment period:

Overall, 321 participants (63.7%) experienced 834 TEAEs during the double-blind treatment period. There was a similar percentage of participants in the ENZ215 group and the Prolia group who experienced TEAEs, severe TEAEs, study treatment-related TEAEs, TESAEs, treatment-emergent AESIs and TEAEs leading to treatment withdrawal.

The most frequently reported TEAEs (those reported in ≥ 5% of participants overall) were in the Infections and Infestations SOC (34.1% of participants); Musculoskeletal and Connective Tissue Disorders SOC (15.7%); Gastrointestinal Disorders SOC (14.5%); Metabolism and Nutrition Disorders SOC (8.5%); Nervous System Disorders SOC (8.1%); Injury, Poisoning and Procedural Complications SOC (6.9%); Investigations (6.2%); and Vascular Disorders SOC (5.2%). At the PT level, the most frequently reported TEAEs (those reported in ≥ 5% of participants overall) were nasopharyngitis (7.9% of participants overall) and upper respiratory tract infection (5.4% overall). The percentage of participants reporting specific TEAEs was generally similar between the two treatment groups; TEAEs with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were COVID-19 (2.0% [one additional participant had a TEAE of coronavirus infection] and 5.2%, respectively), headache (2.4% and 5.2%), upper respiratory tract infection (4.3% and 6.4%) and gastritis (2.0% and 0%).

Most TEAEs were mild or moderate in severity. 45.5% of patients in the ENZ215 group observed mild TEAEs whereas 47.4% patients in the Prolia group observed mild TEAEs. 41.5% of patients in the ENZ215 group observed moderate TEAEs whereas 33.5% of patients in the Prolia group observed moderate TEAEs. There was one SUSAR and one AE/TEAE leading to death, both of which were reported in the Prolia group. Severe TEAEs occurred in five participants (2.0%) in the ENZ215 group (seven severe TEAEs) and five participants (2.0%) in the Prolia group (five severe TEAEs). Severe TEAEs reported in each treatment group were the following (reported in one participant [0.4%] each):

- ENZ215: influenza, pneumonia, staphylococcal bacteraemia, myasthenia gravis, radius fracture, ankle fracture and genital prolapse.
- Prolia: COVID-19, pneumonia, lower limb fracture, myocardial infarction and chromophobe renal cell carcinoma.

All severe TEAEs were assessed as not related to study treatment. All severe TEAEs were also reported as TESAEs, except for the event of pneumonia in the Prolia group. One severe TEAE resulted in treatment withdrawal (pneumonia in the ENZ215 group).

A total of 16 fracture TEAEs were reported in 10 (4.0%) participants in the ENZ215 group and eight fracture TEAEs were reported for seven (2.8%) participants in the Prolia group. Fracture TEAEs reported in more than one participant were radius fracture (five [1.0%] participants overall) and ankle fracture, craniofacial fracture, foot fracture and wrist fracture (each in two [0.4%] participants overall). None of the fracture TEAEs were considered related to study treatment.

Open-label extension period:

Overall, 46 participants (38.3%) experienced 66 TEAEs during the open-label extension period. The proportion of participants in the ENZ215 group and the Prolia group who experienced TEAEs were same. All of the TEAEs were mild or moderate in severity. Two (3.3%) participants in the ENZ215 group had 3 TEAEs

considered related to study treatment. There were no SUSAR, SAE, AE leading to death or AE leading to treatment withdrawal reported during the open-label extension period.

The most frequently reported TEAEs (those reported in ≥ 5% of participants overall) were in the Infections and Infestations SOC (22.5% of participants) and Musculoskeletal and Connective Tissue Disorders SOC (5.8% of participants). At the PT level, the most frequently reported TEAEs (those reported in ≥ 5% of participants overall) were nasopharyngitis (8.3% of participants overall). The percentage of participants reporting specific TEAEs was generally similar between the two treatment groups; TEAEs with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were bronchitis (0% and 3.3%, respectively), pharyngitis, spinal pain and upper respiratory tract infection (each 3.3% and 0%, respectively).

All TEAEs were mild or moderate in severity. No severe or serious TEAEs, TEAE leading to death, or TEAEs leading to treatment withdrawal were observed during the open-label extension period. Overall, two (3.3%) participants had 3 TEAEs which were considered as related to study treatment.

A total of two fracture TEAEs were reported in two (3.3%) participants in the Prolia group. None of the fracture TEAEs were considered related to study treatment.

Table 41. Overview of AEs During the Double-blind and Opel-label Treatment Period (Safety Set)

Adverse Event Categories	ENZ215	Prolia®	Total
Number (%) of Participants	**n (%) #E**	**n (%) #E**	**n (%) #E**
Double-blind Treatment Period	**(N = 253)**	**(N = 251)**	**(N = 504)**
Any AE	166 (65.6) 453	160 (63.7) 412	326 (64.7) 865
Any AE of severe or higher	5 (2.0) 7	6 (2.4) 6	11 (2.2) 13
Any TEAE	164 (64.8) 441	157 (62.5) 393	321 (63.7) 834
Any TEAE of severe or higher	5 (2.0) 7	5 (2.0) 5	10 (2.0) 12
Any SAE	16 (6.3) 23	15 (6.0) 17	31 (6.2) 40
Any TESAE	16 (6.3) 23	15 (6.0) 17	31 (6.2) 40
Any AESI	8 (3.2) 8	7 (2.8) 7	15 (3.0) 15
Any treatment-emergent AESI	8 (3.2) 8	7 (2.8) 7	15 (3.0) 15
Any SUSARs	0	1 (0.4) 1	1 (0.2) 1
Any TEAE related to study treatment	19 (7.5) 25	23 (9.2) 36	42 (8.3) 61
Any TEAE related to non-study treatment	9 (3.6) 9	13 (5.2) 16	22 (4.4) 25
Any TESAE related to study treatment	0	1 (0.4) 1	1 (0.2) 1
Any TESAE related to non-study treatment	0	0	0
Any AE leading to death	0	1 (0.4) 1	1 (0.2) 1
Any TEAE leading to death	0	1 (0.4) 1	1 (0.2) 1
Any AE leading to treatment withdrawal	1 (0.4) 1	2 (0.8) 2	3 (0.6) 3
Any TEAE leading to treatment withdrawal	1 (0.4) 1	2 (0.8) 2	3 (0.6) 3
Open-label Extension Period	**(N = 60)**	**(N = 60)**	**(N = 120)**
Any AE	23 (38.3) 36	23 (38.3) 30	46 (38.3) 66
Any AE of severe or higher	0	0	0
Any TEAE	23 (38.3) 36	23 (38.3) 30	46 (38.3) 66
Any TEAE of severe or higher	0	0	0
Any SAE	0	0	0
Any TESAE	0	0	0
Any AESI	0	0	0
Any treatment emergent AESI	0	0	0
Any SUSARs	0	0	0
Any TEAE related to study treatment	2 (3.3) 3	0	2 (1.7) 3
Any TEAE related to non-study treatment	0	0	0
Any TESAE related to study treatment	0	0	0
Any TESAE related to non-study treatment	0	0	0
Any AE leading to death	0	0	0
Any TEAE leading to death	0	0	0
Any AE leading to treatment withdrawal	0	0	0
Any TEAE leading to treatment withdrawal	0	0	0

Abbreviations: AE = adverse event; AESI = adverse event of special interest; TEAE = treatment-emergent adverse event; SAE = serious adverse event; SUSARs = Suspected unexpected serious adverse reactions; TESAE = treatment-emergent serious adverse event; N = number of participants in the Safety Set; n = number of participants with at least one AE in each category; #E = number of events in each category; % = percentage of participants with at least one AE in each category relative to the total number of participants in the Safety Set.

All participants are counted only once per treatment in each AE category.

TEAE: Date of onset or worsening of the AE is on or after day of dispensation of study drug (until the end of study visit).

Source: Table 14.3.1.1.1 and Table 14.3.1.1.1a.

Table 42. Overview of mild, moderate and severe adverse events during double blind period and open label period (Safety set)

Period: Double blind

Adverse Event Categories	ENZ215 (N=253)		Prolia® (N=251)		Total (N=504)	
Number (%) of Patients	n (%)	#E	n (%)	#E	n (%)	#E
Any AE of mild severity	118 (46.6)	252	123 (49.0)	265	241 (47.8)	517
Any TEAE of mild severity	115 (45.5)	244	119 (47.4)	249	234 (46.4)	493
Any AE of moderate severity	105 (41.5)	194	85 (33.9)	141	190 (37.7)	335
Any TEAE of moderate severity	105 (41.5)	190	84 (33.5)	139	189 (37.5)	329
Any AE of severe severity	5 (2.0)	7	6 (2.4)	6	11 (2.2)	13
Any TEAE of severe severity	5 (2.0)	7	5 (2.0)	5	10 (2.0)	12

Period: Open label

Adverse Event Categories	ENZ215 (N=60)		Prolia® (N=60)		Total (N=120)	
Number (%) of Patients	n (%)	#E	n (%)	#E	n (%)	#E
Any AE of mild severity	13 (21.7)	23	13 (21.7)	13	26 (21.7)	36
Any TEAE of mild severity	13 (21.7)	23	13 (21.7)	13	26 (21.7)	36
Any AE of moderate severity	11 (18.3)	13	12 (20.0)	17	23 (19.2)	30
Any TEAE of moderate severity	11 (18.3)	13	12 (20.0)	17	23 (19.2)	30
Any AE of severe severity	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Any TEAE of severe severity	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0

AE = Adverse event. TEAE = Treatment Emergent Adverse Event. n = Number of patients with at least one AE in each category. #E = Number of events in each category. N = Number of patients in the safety set. % = Percentage of patients with at least one AE in each category relative to the total number of patients in the safety set. All patients are counted only once per treatment in each AE category.

Table 43. TEAEs by SOC and PT Occurring in ⩾ 1% of Participants in Any Group During the Double-blind and Open-label Extension Period (Safety Set)

System Organ Class Preferred Term [1]	ENZ215	Prolia®	Total
Number (%) of Participants	n (%) #E	n (%) #E	n (%) #E
Double-blind Treatment Period	**(N = 253)**	**(N = 251)**	**(N = 504)**
Number of participants with at least one TEAE	164 (64.8) 441	157 (62.5) 393	321 (63.7) 834
Infections and infestations	87 (34.4) 126	85 (33.9) 125	172 (34.1) 251
Nasopharyngitis	18 (7.1) 21	22 (8.8) 24	40 (7.9) 45
Upper respiratory tract infection	11 (4.3) 13	16 (6.4) 19	27 (5.4) 32
COVID-19	5 (2.0) 5	13 (5.2) 13	18 (3.6) 18
Bronchitis	11 (4.3) 12	6 (2.4) 6	17 (3.4) 18
Urinary tract infection	8 (3.2) 8	8 (3.2) 9	16 (3.2) 17
Cystitis	5 (2.0) 5	6 (2.4) 7	11 (2.2) 12
Pharyngitis	6 (2.4) 7	3 (1.2) 3	9 (1.8) 10
Urinary tract infection bacterial	3 (1.2) 4	5 (2.0) 5	8 (1.6) 9
Sinusitis	3 (1.2) 3	4 (1.6) 4	7 (1.4) 7
Respiratory tract infection	1 (0.4) 1	5 (2.0) 6	6 (1.2) 7
Oral herpes	2 (0.8) 2	3 (1.2) 3	5 (1.0) 5
Pulpitis dental	1 (0.4) 1	3 (1.2) 3	4 (0.8) 4
Bronchitis bacterial	3 (1.2) 3	0	3 (0.6) 3
Respiratory tract infection viral	3 (1.2) 4	0	3 (0.6) 4
Musculoskeletal and connective tissue disorders	44 (17.4) 62	35 (13.9) 46	79 (15.7) 108
Arthralgia	9 (3.6) 9	9 (3.6) 13	18 (3.6) 22
Back pain	6 (2.4) 9	6 (2.4) 6	12 (2.4) 15
Osteoarthritis	8 (3.2) 9	4 (1.6) 4	12 (2.4) 13
Spinal pain	5 (2.0) 7	4 (1.6) 4	9 (1.8) 11
Muscle spasms	2 (0.8) 3	3 (1.2) 3	5 (1.0) 6
Pain in extremity	3 (1.2) 3	2 (0.8) 2	5 (1.0) 5
Gastrointestinal disorders	38 (15.0) 46	35 (13.9) 42	73 (14.5) 88
Diarrhoea	6 (2.4) 6	6 (2.4) 6	12 (2.4) 12
Abdominal pain upper	2 (0.8) 2	5 (2.0) 6	7 (1.4) 8
Chronic gastritis	2 (0.8) 2	3 (1.2) 3	5 (1.0) 5
Constipation	3 (1.2) 3	2 (0.8) 3	5 (1.0) 6
Gastritis	5 (2.0) 6	0	5 (1.0) 6
Gastrooesophageal reflux disease	3 (1.2) 3	2 (0.8) 2	5 (1.0) 5
Toothache	3 (1.2) 3	2 (0.8) 2	5 (1.0) 5
Nausea	0	3 (1.2) 3	3 (0.6) 3
Metabolism and nutrition disorders	24 (9.5) 26	19 (7.6) 20	43 (8.5) 46
Hypocalcaemia	7 (2.8) 7	6 (2.4) 6	13 (2.6) 13
Vitamin D deficiency	6 (2.4) 6	4 (1.6) 4	10 (2.0) 10
Hypercalcaemia	2 (0.8) 2	4 (1.6) 4	6 (1.2) 6
Hyperlipidaemia	3 (1.2) 3	1 (0.4) 1	4 (0.8) 4
Nervous system disorders	22 (8.7) 30	19 (7.6) 25	41 (8.1) 55

Headache	6 (2.4) 9	13 (5.2) 16	19 (3.8) 25
Radiculopathy	3 (1.2) 3	0	3 (0.6) 3
Injury, poisoning and procedural complications	19 (7.5) 29	16 (6.4) 18	35 (6.9) 47
Radius fracture	4 (1.6) 5	1 (0.4) 1	5 (1.0) 6
Meniscus injury	3 (1.2) 4	0	3 (0.6) 4
Investigations	12 (4.7) 12	19 (7.6) 21	31 (6.2) 33
Vitamin D decreased	6 (2.4) 6	6 (2.4) 6	12 (2.4) 12
Vascular disorders	12 (4.7) 12	14 (5.6) 16	26 (5.2) 28
Hypertension	9 (3.6) 9	12 (4.8) 14	21 (4.2) 23
Skin and subcutaneous tissue disorders	11 (4.3) 12	8 (3.2) 10	19 (3.8) 22
General disorders and administration site conditions	10 (4.0) 11	7 (2.8) 8	17 (3.4) 19
Fatigue	3 (1.2) 3	2 (0.8) 2	5 (1.0) 5
Respiratory, thoracic and mediastinal disorders	11 (4.3) 12	5 (2.0) 5	16 (3.2) 17
Cardiac disorders	7 (2.8) 10	7 (2.8) 10	14 (2.8) 20
Renal and urinary disorders	5 (2.0) 7	9 (3.6) 9	14 (2.8) 16
Eye disorders	8 (3.2) 10	5 (2.0) 9	13 (2.6) 19
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	4 (1.6) 6	8 (3.2) 9	12 (2.4) 15
Ear and labyrinth disorders	5 (2.0) 7	5 (2.0) 5	10 (2.0) 12
Vertigo	3 (1.2) 3	0	3 (0.6) 3
Reproductive system and breast disorders	5 (2.0) 5	5 (2.0) 6	10 (2.0) 11
Immune system disorders	6 (2.4) 7	3 (1.2) 3	9 (1.8) 10
Endocrine disorders	4 (1.6) 4	2 (0.8) 2	6 (1.2) 6
Hepatobiliary disorders	4 (1.6) 4	2 (0.8) 2	6 (1.2) 6
Open-label Extension Period	**(N = 60)**	**(N = 60)**	**(N = 120)**
Number of patients with at least one TEAE	23 (38.3) 36	23 (38.3) 30	46 (38.3) 66
Infections and infestations	14 (23.3) 15	13 (21.7) 13	27 (22.5) 28
Nasopharyngitis	5 (8.3) 6	5 (8.3) 5	10 (8.3) 11
Bronchitis	0 (0.0) 0	2 (3.3) 2	2 (1.7) 2
COVID-19	1 (1.7) 1	1 (1.7) 1	2 (1.7) 2
Pharyngitis	2 (3.3) 2	0 (0.0) 0	2 (1.7) 2
Upper respiratory tract infection	2 (3.3) 2	0 (0.0) 0	2 (1.7) 2
Gastroenteritis	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Herpes zoster	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Influenza	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Lyme disease	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Pneumonia	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Rhinitis	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Sinusitis	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1

Tracheobronchitis	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Viral infection	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Musculoskeletal and connective tissue disorders	5 (8.3) 5	2 (3.3) 3	7 (5.8) 8
Myalgia	1 (1.7) 1	1 (1.7) 1	2 (1.7) 2
Osteoarthritis	1 (1.7) 1	1 (1.7) 1	2 (1.7) 2
Spinal pain	2 (3.3) 2	0 (0.0) 0	2 (1.7) 2
Rotator cuff syndrome	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Shoulder girdle pain	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Metabolism and nutrition disorders	2 (3.3) 2	3 (5.0) 3	5 (4.2) 5
Hypomagnesaemia	1 (1.7) 1	1 (1.7) 1	2 (1.7) 2
Dyslipidaemia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Hypercholesterolaemia	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Hypertriglyceridaemia	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Injury, poisoning and procedural complications	1 (1.7) 1	3 (5.0) 3	4 (3.3) 4
Lumbar vertebral fracture	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Meniscus injury	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Thoracic vertebral fracture	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Tooth fracture	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Eye disorders	3 (5.0) 3	0 (0.0) 0	3 (2.5) 3
Blepharochalasis	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Chalazion	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Glaucoma	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Respiratory, thoracic and mediastinal disorders	1 (1.7) 1	2 (3.3) 2	3 (2.5) 3
Chronic obstructive pulmonary disease	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Dyspnoea	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Upper respiratory tract inflammation	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Gastrointestinal disorders	1 (1.7) 1	1 (1.7) 1	2 (1.7) 2
Abdominal pain	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Diarrhoea	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2 (3.3) 2	0 (0.0) 0	2 (1.7) 2
Benign neoplasm of conjunctiva	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Uterine leiomyoma	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Blood and lymphatic system disorders	1 (1.7) 2	0 (0.0) 0	1 (0.8) 2
Leukopenia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Neutropenia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Cardiac disorders	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Atrioventricular block first degree	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Endocrine disorders	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1

Autoimmune thyroiditis	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Hepatobiliary disorders	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Hepatic steatosis	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Immune system disorders	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Drug hypersensitivity	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Investigations	0 (0.0) 0	1 (1.7) 2	1 (0.8) 2
Alanine aminotransferase increased	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Aspartate aminotransferase increased	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Nervous system disorders	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Dizziness	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Psychiatric disorders	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Anxiety	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Skin and subcutaneous tissue disorders	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Alopecia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1

Abbreviations: COVID-19 = coronavirus disease-2019; MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event; N = number of participants in safety set; n = number of participants with at least one TEAE in each category; #E = number of events in each category; % = percentage of participants with at least one TEAE in each category relative to the total number of participants in the Safety Set.

All participants are counted only once per treatment in each TEAE category.

[1] Within a SOC, participants may have reported more than one PT.

Sorting order: descending by the number of participants of overall group by SOC and descending by the number of participants of overall group by PT. In case of ties, ascending order by PT Code is applied.

MedDRA Version 26.1.

Source: Table 14.3.1.1.2 and Table 14.3.1.1.2a.

Table 44. TEAEs of Fracture During the Double-blind and Open-label Extension Period (Safety Set)

Preferred Term [1]	ENZ215	Prolia®	Total
Number (%) of Participants	n (%) #E	n (%) #E	n (%) #E
Double-blind Treatment Period	(N = 253)	(N = 251)	(N = 504)
Radius fracture	4 (1.6) 5	1 (0.4) 1	5 (1.0) 6
Ankle fracture	1 (0.4) 1	1 (0.4) 1	2 (0.4) 2
Craniofacial fracture	1 (0.4) 1	1 (0.4) 1	2 (0.4) 2
Foot fracture	1 (0.4) 2	1 (0.4) 1	2 (0.4) 3
Wrist fracture	2 (0.8) 3	0 (0.0) 0	2 (0.4) 3
Forearm fracture	0 (0.0) 0	1 (0.4) 1	1 (0.2) 1
Fracture displacement	1 (0.4) 1	0 (0.0) 0	1 (0.2) 1
Hand fracture	0 (0.0) 0	1 (0.4) 1	1 (0.2) 1
Humerus fracture	1 (0.4) 1	0 (0.0) 0	1 (0.2) 1
Lower limb fracture	0 (0.0) 0	1 (0.4) 1	1 (0.2) 1
Spinal compression fracture	1 (0.4) 1	0 (0.0) 0	1 (0.2) 1
Thoracic vertebral fracture	0 (0.0) 0	1 (0.4) 1	1 (0.2) 1
Ulna fracture	1 (0.4) 1	0 (0.0) 0	1 (0.2) 1
Open-label Extension Period	(N = 60)	(N = 60)	(N = 120)
Lumbar vertebral fracture	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1
Thoracic vertebral fracture	0 (0.0) 0	1 (1.7) 1	1 (0.8) 1

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; TEAE = treatment-emergent adverse event; N = number of participants in safety set; n = number of participants with at least one TEAE in each category; #E = number of events in each category; % = percentage of participants with at least one TEAE in each category relative to the total number of participants in the Safety Set.

All participants are counted only once per treatment in each TEAE category.

[1] Participants may have reported more than one PT.

Sorting order: descending by the number of participants of overall group by PT. In case of ties, ascending order by PT Code is applied.

MedDRA Version 26.1.

Source: Table 14.3.1.1.2 and Table 14.3.1.1.2a.

2.5.8.3. Adverse drug reactions

2.5.8.3.1. Healthy male population (Study ALK22/ENZ215-DEN1)

Overall, 15 (7.3%) subjects experienced at least 1 TEAE considered as related to the study treatment, where the incidence was similar in all treatment groups. The most commonly reported treatment-related TEAEs by SOC were metabolism and nutrition disorders (8 [3.9%] subjects) and gastrointestinal disorders (4 [1.9% subjects). The most commonly reported treatment-related TEAEs by PT was hypocalcemia (8 [3.9%] subjects); all other treatment-related TEAEs were reported by 1 subject each.

Table 45. Summary of Treatment-related Adverse Events by System Organ Class and Preferred Term (Safety Analysis Set)

	ENZ215 (N=68)		US-Prolia® (N=69)		EU-Prolia® (N=69)		Total (N=206)	
System Organ Class/ Preferred Term	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n	Subjects n(%)	Events n
Number of subjects with related TEAEs	5 (7.4)	5	4 (5.8)	4	6 (8.7)	8	15 (7.3)	17
Gastrointestinal disorders	2 (2.9)	2	0	0	2 (2.9)	2	4 (1.9)	4
Abdominal pain lower	1 (1.5)	1	0	0	0	0	1 (0.5)	1
Aphthous ulcer	1 (1.5)	1	0	0	0	0	1 (0.5)	1
Hyperaesthesia teeth	0	0	0	0	1 (1.4)	1	1 (0.5)	1
Toothache	0	0	0	0	1 (1.4)	1	1 (0.5)	1
General disorders and administration site conditions	0	0	0	0	1 (1.4)	1	1 (0.5)	1
Administration site bruise	0	0	0	0	1 (1.4)	1	1 (0.5)	1
Metabolism and nutrition disorders	3 (4.4)	3	2 (2.9)	2	3 (4.3)	3	8 (3.9)	8
Hypocalcaemia	3 (4.4)	3	2 (2.9)	2	3 (4.3)	3	8 (3.9)	8
Musculoskeletal and connective tissue disorders	0	0	0	0	2 (2.9)	2	2 (1.0)	2
Arthralgia	0	0	0	0	1 (1.4)	1	1 (0.5)	1
Musculoskeletal pain	0	0	0	0	1 (1.4)	1	1 (0.5)	1
Nervous system disorders	0	0	2 (2.9)	2	0	0	2 (1.0)	2
Dizziness	0	0	1 (1.4)	1	0	0	1 (0.5)	1
Headache	0	0	1 (1.4)	1	0	0	1 (0.5)	1

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.
Note(s): System organ class and preferred term are from the MedDRA dictionary, Version 25.0 for subjects of site 01 and from MedDRA dictionary, Version 26.1 for subjects of site 02 and 03.
The numbers of subjects within each column cannot be added because a subject may have had more than 1 adverse event.
A subject experiencing multiple occurrences of an adverse event was counted, at most, once per system organ class and preferred term.
Related TEAEs are defined as those assessed as related to study drug, or those for which the relationship is unknown or missing.
Source: Table 14.3.1.3.

2.5.8.3.2. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

Double-blind treatment period:

Treatment-related TEAEs were reported in 19 participants (7.5%) in the ENZ215 group and 23 participants (9.2%) in the Prolia group. Hypocalcaemia was the most frequently reported treatment-related TEAE, occurring in seven participants (2.8%) in the ENZ215 group and five participants (2.0%) in the Prolia group.

No injection site reactions were reported in the ENZ215 group.

Two participants in the Prolia group experienced injection site reactions, including one mild TEAE of injection site erythema and one moderate TEAE of injection site haemorrhage. Both injection site reactions were non-serious and assessed as related to study treatment.

Open-label extension period:

Overall, three treatment-related TEAEs were reported in two participants (3.3%) in the ENZ215 group. One participant had two mild treatment-related TEAEs of leukopenia and neutropenia; and one participant had a moderate treatment-related TEAE of alopecia.

No injection site reactions were reported during the open-label extension period.

Table 46. Treatment-Related TEAEs by SOC and PT During the Double-blind and Open-label Extension Period (Safety Set)

System Organ Class Preferred Term [1]	ENZ215	Prolia®	Total
Number (%) of Participants	**n (%) #E**	**n (%) #E**	**n (%) #E**
Double-blind Treatment Period	**(N = 253)**	**(N = 251)**	**(N = 504)**
Musculoskeletal and connective tissue disorders	5 (2.0) 6	9 (3.6) 11	14 (2.8) 17
Myalgia	2 (0.8) 2	1 (0.4) 1	3 (0.6) 3
Back pain	0	2 (0.8) 2	2 (0.4) 2
Bone pain	0	2 (0.8) 2	2 (0.4) 2
Musculoskeletal pain	1 (0.4) 1	1 (0.4) 1	2 (0.4) 2
Pain in extremity	0	2 (0.8) 2	2 (0.4) 2
Arthralgia	0	1 (0.4) 1	1 (0.2) 1
Joint swelling	0	1 (0.4) 1	1 (0.2) 1
Pain in jaw	1 (0.4) 1	0	1 (0.2) 1
Spinal osteoarthritis	0	1 (0.4) 1	1 (0.2) 1
Spinal pain	1 (0.4) 2	0	1 (0.2) 2
Metabolism and nutrition disorders	7 (2.8) 7	5 (2.0) 5	12 (2.4) 12
Hypocalcaemia	7 (2.8) 7	5 (2.0) 5	12 (2.4) 12
Infections and infestations	3 (1.2) 3	5 (2.0) 6	8 (1.6) 9
Cystitis	1 (0.4) 1	1 (0.4) 2	2 (0.4) 3
Bronchitis mycoplasmal	0	1 (0.4) 1	1 (0.2) 1
Nasopharyngitis	0	1 (0.4) 1	1 (0.2) 1
Oral herpes	0	1 (0.4) 1	1 (0.2) 1
Respiratory tract infection	0	1 (0.4) 1	1 (0.2) 1
Sinusitis bacterial	1 (0.4) 1	0	1 (0.2) 1
Skin infection	1 (0.4) 1	0	1 (0.2) 1
General disorders and administration site conditions	2 (0.8) 3	5 (2.0) 6	7 (1.4) 9
Fatigue	2 (0.8) 2	2 (0.8) 2	4 (0.8) 4
Asthenia	0	1 (0.4) 1	1 (0.2) 1
Chills	1 (0.4) 1	0	1 (0.2) 1
Injection site erythema	0	1 (0.4) 1	1 (0.2) 1
Injection site haemorrhage	0	1 (0.4) 1	1 (0.2) 1
Malaise	0	1 (0.4) 1	1 (0.2) 1
Skin and subcutaneous tissue disorders	4 (1.6) 4	1 (0.4) 1	5 (1.0) 5
Alopecia	2 (0.8) 2	1 (0.4) 1	3 (0.6) 3
Granuloma annulare	1 (0.4) 1	0	1 (0.2) 1
Night sweats	1 (0.4) 1	0	1 (0.2) 1
Gastrointestinal disorders	1 (0.4) 1	3 (1.2) 3	4 (0.8) 4
Nausea	0	2 (0.8) 2	2 (0.4) 2
Aphthous ulcer	1 (0.4) 1	0	1 (0.2) 1
Large intestine polyp	0	1 (0.4) 1	1 (0.2) 1
Nervous system disorders	1 (0.4) 1	3 (1.2) 3	4 (0.8) 4
Headache	0	3 (1.2) 3	3 (0.6) 3
Paraesthesia	1 (0.4) 1	0	1 (0.2) 1
Ear and labyrinth disorders	0	1 (0.4) 1	1 (0.2) 1
Tinnitus	0	1 (0.4) 1	1 (0.2) 1
Open-label Extension Period	**(N = 60)**	**(N = 60)**	**(N = 60)**
Blood and lymphatic system disorders	1 (1.7) 2	0 (0.0) 0	1 (0.8) 2
Leukopenia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Neutropenia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Skin and subcutaneous tissue disorders	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1
Alopecia	1 (1.7) 1	0 (0.0) 0	1 (0.8) 1

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event; N = number of participants in safety set; n = number of participants with at least one TEAE in each category; #E = number of events in each category; % = percentage of participants with at least one TEAE in each category relative to the total number of participants in the Safety Set.

All participants are counted only once per treatment in each TEAE category.

[1] Within an SOC, patients may have reported more than one PT.

2.5.8.4. Serious adverse event/deaths/other significant events

2.5.8.4.1. Healthy male population (Study ALK22/ENZ215-DEN1)

Deaths

There were no deaths in this study.

SAE

One subject in EU-sourced Prolia group experienced treatment-emergent SAE of ligament rupture which resulted in hospitalization and was considered by the investigator as not related to the study treatment. No subject in ENZ215 and US-sourced Prolia groups experienced treatment-emergent SAE.

AESI

There was no collection or definition of Adverse event of special interest (AESI).

Hypocalcemia which is a potential risk with the Prolia treatment was observed in <5% of subjects across treatments (ENZ215: 3 subjects [4.4%], EU-sourced Prolia: 3 subjects [4.3%], US-sourced Prolia: 2 subjects [2.9%]); all the events were mild or moderate in intensity and were resolved.

2.5.8.4.2. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

Deaths

Double-blind treatment period:

One death was reported during the double-blind treatment period. The death occurred in the Prolia group on study day 385 and was caused by a TESAE of COVID-19 that was assessed as not related to study treatment.

Open-label extension period:

No death was reported during the open-label extension period.

SAE

Double-blind treatment period:

All SAEs were treatment-emergent. The overall frequency of TESAEs was 6.2% and was similar in the ENZ215 group and the Prolia group. Most TESAEs were in the Injury, Poisoning and Procedural Complications SOC and consisted of various types of fractures. TESAEs occurring in more than one participant in the ENZ215 group were radius fracture (three participants) and wrist fracture (two participants). All TESAEs in the Prolia group were reported in one participant each.

Only one TESAE was assessed as related to study treatment (SUSAR) and was an event of cystitis in the Prolia group.

Open-label extension period:

No SAE or SUSAR were reported during the open-label extension period.

Table 47. Serious Treatment-Emergent AEs by SOC and PT During the Double-blind Treatment Period (Safety Set)

System Organ Class Preferred Term [1]	ENZ215 (N = 253)	Prolia® (N = 251)	Total (N = 504)
Number (%) of Participants	**n (%) #E**	**n (%) #E**	**n (%) #E**
Number of participants with at least one TESAE	16 (6.3) 23	15 (6.0) 17	31 (6.2) 40
Injury, poisoning and procedural complications	8 (3.2) 11	6 (2.4) 6	14 (2.8) 17
Radius fracture	3 (1.2) 4	1 (0.4) 1	4 (0.8) 5
Ankle fracture	1 (0.4) 1	1 (0.4) 1	2 (0.4) 2
Wrist fracture	2 (0.8) 2	0	2 (0.4) 2
Craniofacial fracture	0	1 (0.4) 1	1 (0.2) 1
Foot fracture	0	1 (0.4) 1	1 (0.2) 1
Forearm fracture	0	1 (0.4) 1	1 (0.2) 1
Fracture displacement	1 (0.4) 1	0	1 (0.2) 1
Humerus fracture	1 (0.4) 1	0	1 (0.2) 1
Lower limb fracture	0	1 (0.4) 1	1 (0.2) 1
Meniscus injury	1 (0.4) 1	0	1 (0.2) 1
Ulna fracture	1 (0.4) 1	0	1 (0.2) 1
Infections and infestations	3 (1.2) 4	2 (0.8) 2	5 (1.0) 6
COVID-19	0	1 (0.4) 1	1 (0.2) 1
Cystitis	0	1 (0.4) 1	1 (0.2) 1
Diverticulitis	1 (0.4) 1	0	1 (0.2) 1
Influenza	1 (0.4) 1	0	1 (0.2) 1
Pneumonia	1 (0.4) 1	0	1 (0.2) 1
Staphylococcal bacteraemia	1 (0.4) 1	0	1 (0.2) 1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (0.4) 1	4 (1.6) 4	5 (1.0) 5
Adenocarcinoma of colon	1 (0.4) 1	0	1 (0.2) 1
Basal cell carcinoma	0	1 (0.4) 1	1 (0.2) 1
Breast cancer	0	1 (0.4) 1	1 (0.2) 1
Chromophobe renal cell carcinoma	0	1 (0.4) 1	1 (0.2) 1
Meningioma benign	0	1 (0.4) 1	1 (0.2) 1
Cardiac disorders	2 (0.8) 2	2 (0.8) 2	4 (0.8) 4
Angina pectoris	0	1 (0.4) 1	1 (0.2) 1
Aortic valve stenosis	1 (0.4) 1	0	1 (0.2) 1
Myocardial infarction	0	1 (0.4) 1	1 (0.2) 1
Pericarditis	1 (0.4) 1	0	1 (0.2) 1
Nervous system disorders	2 (0.8) 2	1 (0.4) 1	3 (0.6) 3
Ischaemic cerebral infarction	1 (0.4) 1	0	1 (0.2) 1
Myasthenia gravis	1 (0.4) 1	0	1 (0.2) 1
Thoracic radiculopathy	0	1 (0.4) 1	1 (0.2) 1
Gastrointestinal disorders	1 (0.4) 1	0	1 (0.2) 1
Gastrooesophageal reflux disease	1 (0.4) 1	0	1 (0.2) 1
Hepatobiliary disorders	0	1 (0.4) 1	1 (0.2) 1
Bile duct stone	0	1 (0.4) 1	1 (0.2) 1
Investigations	0	1 (0.4) 1	1 (0.2) 1
Red blood cells urine positive	0	1 (0.4) 1	1 (0.2) 1
Musculoskeletal and connective tissue disorders	1 (0.4) 1	0	1 (0.2) 1
SAPHO syndrome	1 (0.4) 1	0	1 (0.2) 1
Reproductive system and breast disorders	1 (0.4) 1	0	1 (0.2) 1
Genital prolapse	1 (0.4) 1	0	1 (0.2) 1

Abbreviations: COVID-19 = coronavirus disease 2019; MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; SAE = serious adverse event; SAPHO = synovitis, acne, pustulosis, hyperostosis, osteitis syndrome; SOC = system organ class; TESAE = treatment-emergent serious adverse event; N = number of participants in Safety Set; n = number of participants with at least one TESAE in each category; #E = number of events in each category; % = percentage of participants with at least one TESAE in each category relative to the total number of participants in the Safety Set. All participants are counted only once per treatment in each TESAE category.

[1] Within an SOC, participants may have reported more than one PT.

AESI

According to the protocol, AEs of special interest (AESI) include hypocalcemia, ONJ, atypical femoral fracture, fracture healing complications, severe infection (including skin infection) not leading to hospitalization, hypersensitivity leading to emergency room visit, and potential Hy's law. Any AESI should be reported within 24 hours to Parexel. Clinical monitoring of calcium levels will be done at each pre-defined visit. If any patient presents with suspected symptoms of hypocalcemia during the study, calcium levels should be measured. If a patient develops hypocalcemia over the course of the study, calcium and/or vitamin D supplementation may be adjusted per Investigator's medical judgment until the serum calcium concentration returns to the normal range. In addition, patients will be encouraged to report symptoms indicative of hypocalcemia.

Double-blind treatment period:

AESIs occurred in eight participants (3.2%) in the ENZ215 group and seven participants (2.8%) in the Prolia group (all were treatment-emergent). Hypocalcaemia was the most frequently reported AESI, occurring in seven participants (2.8%) in the ENZ215 group and six participants (2.4%) in the Prolia group. All TEAEs of hypocalcaemia were non-serious and mild in severity, except for one event in the ENZ215 group that was moderate in severity. All TEAEs of hypocalcaemia were assessed as related to study treatment except for one event in the Prolia group that was assessed as not related.

Aside from hypocalcaemia, two other AESIs were reported - a TESAE of fracture displacement (moderate severity) in one participant in the ENZ215 group and a non-serious TEAE of pneumonia (severe) in one participant in the Prolia group; both events were assessed as not related to study treatment. All the AESIs were recovered except for one AESI of hypocalcaemia in the ENZ215 group that was reported as ongoing.

Open-label extension period:

No AESI was reported during the open-label extension period.

2.5.8.5. ADRs of special interest, serious ADRs and deaths causally related to the medicinal product.

2.5.8.5.1. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

SAE

Double-blind treatment period:

Only one TESAE was assessed as related to study treatment (SUSAR) and was an event of cystitis in the Prolia group.

AESI

Double-blind treatment period:

AESIs occurred in eight participants (3.2%) in the ENZ215 group and seven participants (2.8%) in the Prolia group (all were treatment-emergent). Hypocalcaemia was the most frequently reported AESI, occurring in seven participants (2.8%) in the ENZ215 group and six participants (2.4%) in the Prolia group. All TEAEs of hypocalcaemia were non-serious and mild in severity, except for one event in the ENZ215 group that was moderate in severity. All TEAEs of hypocalcaemia were assessed as related to study treatment except for one event in the Prolia group that was assessed as not related.

Table 48. Treatment-emergent adverse events by system organ class, preferred term and relationship to study treatment during double blind phase (Safety set)

System Organ Class Preferred Term [1]	Relationship	ENZ215 (N=253)		Prolia® (N=251)		Total (N=504)	
Number (%) of Patients		n (%)	#E	n (%)	#E	n (%)	#E
Number of patients with at least one TEAE		164 (64.8)	441	157 (62.5)	393	321 (63.7)	834
Infections and infestations	Related	3 (1.2)	3	5 (2.0)	6	8 (1.6)	9
	Non-Related	84 (33.2)	123	80 (31.9)	119	164 (32.5)	242
Nasopharyngitis	Related	0		1 (0.4)	1	1 (0.2)	1
	Non-Related	18 (7.1)	21	21 (8.4)	23	39 (7.7)	44
Upper respiratory tract infection	Related	0		0		0	
	Non-Related	11 (4.3)	13	16 (6.4)	19	27 (5.4)	32

N = Number of patients in safety set; n = Number of patients with at least one TEAE in each category; TEAE = Treatment Emergent Adverse Event; #E = Number of events in each category; % = Percentage of patients with at least one TEAE in each category relative to the total number of patients in the safety set. All patients are counted only once per treatment in each TEAE category.
[1] Within a System Organ Class, patients may have reported more than one Preferred Term.
Sorting order: descending by the number of patients of overall group by System Organ Class and descending by the number of patients of overall group by Preferred Term. In case of ties, ascending order by Preferred Term Code is applied.
MedDRA Version 26.1
Source: Listing 16.2.7.1

2.5.8.6. Laboratory findings

2.5.8.6.1. Healthy male population (Study ALK22/ENZ215-DEN1)

Haematology

There were no clinically meaningful changes in mean values from baseline to end of study (Day 270) for any haematology parameters within the treatment groups. No treatment group differences were noted in the mean change from baseline for any haematology parameter. None of the subjects were reported with haematology abnormalities assessed as clinically significant by the Investigator. There were no TEAEs related to haematology laboratory results

Serum Chemistry

There were no clinically meaningful changes in mean values from baseline to each time point for any serum chemistry parameters within the treatment groups. No treatment group differences were noted in the mean change from baseline for any serum chemistry parameter. Only 2 subjects were reported with serum chemistry abnormalities assessed as clinically significant by the Investigator at end of study Visit (Day 270). Two subjects in US-Prolia group reported 2 TEAEs (aspartate aminotransferase increased and alanine aminotransferase increased). Both these TEAEs were moderate in intensity, considered as not related to the study treatment, and were resolved. There were no serious TEAEs related to serum chemistry laboratory results.

Urinalysis

There were no clinically meaningful changes in mean values from baseline to end of study (Day 270) for any urinalysis parameters within the treatment groups. No treatment group differences were noted in the mean change from baseline for any urinalysis parameter. None of the subjects were reported with urinalysis

abnormalities assessed as clinically significant by the Investigator. There were no TEAEs related to urinalysis laboratory results.

Vital Signs

There were no clinically meaningful changes in mean values from baseline to each time point for any vital sign parameter.

Individual Clinically Relevant Abnormalities

Two abnormal vital sign assessments were reported as TEAEs in 2 subjects. These included a moderate event of hypertension, and a mild event of blood pressure increased. Both these AEs were considered as not related to the study treatment. The AE of hypertension was resolved, and blood pressure increased was ongoing at the end of the study. There were no serious TEAEs related to vital signs results.

Physical Examination Findings

None of the subjects were reported with physical examination abnormalities assessed as clinically significant by the Investigator.

Electrocardiogram Assessments

All the abnormal ECG findings were assessed as not clinically significant by the Investigators. No adverse events were reported based on the ECG measurements. Two subjects each in ENZ215 and EU-Prolia groups and 1 subject in US-Prolia group were reported with clinically noteworthy ECG values (> 30 ms and ≤ 60 ms) which were outliers.

Oral Examination

None of the subjects were reported with oral examination abnormalities assessed as clinically significant by the Investigator.

2.5.8.6.2. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

Haematology

Double-blind treatment period:

Shifts from normal at baseline to low at worst post-baseline assessment with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were observed for erythrocytes (13.8% and 9.2%, respectively), haemoglobin (6.3% and 3.2%), and platelets (4.7% and 1.6%).

Open-label extension period:

Shifts from normal at baseline to low at worst post-baseline assessment with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were observed for haemoglobin (5.0% and 1.7%). Shifts from normal at baseline to high at worst post-baseline assessment with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were observed for leukocytes and neutrophils (0 and 3.3%).

Serum Chemistry

Double-blind treatment period:

Shifts in serum chemistry parameters from normal at baseline to low or high at worst post-baseline assessment with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were observed for the following:

Liver function tests:

- ALT shift to high: ENZ215: seven (2.8%); Prolia: 13 (5.2%)

Renal function tests:

- BUN shift to high: ENZ215: five (2.0%); Prolia: 15 (6.0%)
- eGFR shift to low: ENZ215: 38 (15.0%); Prolia: 46 (18.3%)

Calcium and mineral metabolism tests:

- Calcium corrected shift to low: ENZ215: 49 (19.4%); Prolia: 54 (21.5%)
- Magnesium shift to low: ENZ215: 12 (4.7%); Prolia: 22 (8.8%)

Shifts in other evaluated serum chemistry parameters from normal at baseline to low or high at worst post-baseline assessment occurred at a similar frequency in the ENZ215 group and the Prolia group.

Open-label extension period:

Shifts in serum chemistry parameters from normal at baseline to low or high at worst post-baseline assessment with a ≥ 2% difference in frequency between the ENZ215 group and the Prolia group were observed for the following:

Liver function tests:

- AST shift to high: ENZ215: 0; Prolia: 2 (3.3%)

Calcium and mineral metabolism tests:

- Magnesium shift to low: ENZ215: 0; Prolia: 4 (6.7%)
- Sodium shift to low: ENZ215: 1 (1.7); Prolia: 4 (6.7%)

Glucose shift to high: ENZ215: 0; Prolia: 2 (3.3%)

Shifts in other evaluated serum chemistry parameters from normal at baseline to low or high at worst post-baseline assessment occurred at a similar frequency in the ENZ215 group and the Prolia group.

Urinalysis

There were no major or consistent post-screening differences in the percentage of participants in the ENZ215 group and the Prolia group for urinalysis categorical measurements (i.e., amorphous crystals, calcium oxalate crystals, crystals, epithelial cells, glucose clearance, ketones, nitrite, sample aspect, bilirubin, erythrocytes, haemoglobin, leukocytes, and urobilinogen) except for bacteria negative (41.7% vs 31.7%) and colour-yellow (35% vs 45%) at Visit 11.

Clinically Meaningful Laboratory Abnormalities

Clinically significant abnormal laboratory findings were defined as those not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. Any finding meeting these criteria should have been reported as an AE.

Double-blind treatment period:

Vitamin D decreased was the most frequently reported PT, reported by 2.4% of participants in each group. All TEAEs in the Investigations SOC were mild or moderate in severity and were non-serious, except for the TEAE of red blood cells urine positive in the Prolia group, which was reported as an SAE (this participant also experienced a SUSAR of cystitis). None of the TEAEs in the Investigations SOC associated with a laboratory finding were assessed as related to study treatment.

Open-label extension period:

At SOC level, the most frequently reported AEs with clinically meaningful abnormalities were of Metabolism and nutrition disorders (3.3% and 5.0% in the ENZ215 and Prolia group, respectively). One participant (1.7%) in the ENZ215 group had reported two AEs of leukopenia and neutropenia and one participant (1.7%) in the Prolia group had reported two AEs of liver enzyme increased. A participant in the Prolia group had high ALT and AST at 194 U/L and 153 U/L, respectively. The event was considered unrelated to study treatment and resolved later. A participant in the ENZ215 group had low leukocytes and neutrophil count of 2.5×109/L and 0.6×109/L, respectively which were considered related to study treatment and was ongoing at the time of report, but no treatment was needed.

Vital Signs

Double-blind treatment period:

There were no major differences between the ENZ215 group and the Prolia group in mean changes from baseline to Visit 9/Month 12 in vital sign parameters (i.e., weight, height, temperature, systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate). The most frequently reported TEAE related to vital signs was hypertension in nine participants (3.6%) in the ENZ215 group and 12 participants (4.8%) in the Prolia group. Blood pressure increased was reported in one participant (0.4%) in the ENZ215 group and two participants (0.8%) in the Prolia group. Other vital sign-related TEAEs (blood pressure systolic increased, white coat hypertension, sinus tachycardia, supraventricular tachycardia, and pyrexia) occurred in one participant each. All of these TEAEs were mild or moderate in severity, non-serious, and assessed as not related to study treatment.

Open-label extension period:

There were no clinically meaningful findings in the vital sign measurements related to safety in the open-label extension period of this study.

Electrocardiograms

Double-blind treatment period:

Shifts in 12-lead ECG results from normal at baseline to abnormal, clinically significant at worst post-baseline occurred in one participant (0.4%) in both the ENZ215 group and the Prolia group. One participant in the ENZ215 group had a mild, non-serious, non-related AE of atrioventricular block first degree reported during the screening period, which was ongoing at the time of the report. One participant in the Prolia group had a mild, non-serious, non-related TEAE of atrioventricular block first degree, reported during the double-blind treatment period, which was ongoing at the time of the report.

Open-label extension period:

The participant in the Prolia group had a mild, non-serious, non-related TEAE of atrioventricular block first degree reported during the open-label extension period, that was ongoing at the time of the report.

Physical Examination Findings

Shifts in physical examination results from normal at baseline to abnormal, clinically significant at worst post-baseline during the double-blind treatment period and open-label extension period are presented below by body system (reported as number and percentage of participants in the ENZ215 group and the Prolia group, respectively):

- Double-blind treatment period:
    - General appearance: three (1.2%) and four (1.6%)
    - Oro-dental: five (2.0%) and zero
    - Skin: three (1.2%) and one (0.4%)
    - Gastrointestinal tract: zero and two (0.8%)
    - Cardiovascular: one (0.4%) and zero
    - Nervous system: one (0.4%) and zero.
- Open-label extension period
    - Oro-dental: zero and one (1.7%)

Shifts in physical examination results from abnormal, not clinically significant at baseline to abnormal, clinically significant at worst post-baseline during the double-blind treatment period were the following (reported as number and percentage of participants in the ENZ215 group and the Prolia group, respectively):

- General appearance: zero and one (0.4%)
- Oro-dental: one (0.4%) and zero
- Skin: two (0.8%) and zero
- Cardiovascular: zero and one (0.4%)

In the open-label extension period, none of the participants had shift in physical examination results from abnormal, not clinically significant at baseline to abnormal, clinically significant at worst post baseline.

The TEAEs reported for participants with shifts in physical examination results from normal or abnormal, not clinically significant at baseline to abnormal, clinically significant post-baseline during the double-blind treatment period were the following:

General appearance:

- ENZ215: Two of the TEAEs were moderate and non-serious (eyelid ptosis and joint injury) and one was severe and an SAE (radius fracture). None were assessed as related to the study treatment.
- Prolia: Three of the TEAEs were moderate and non-serious (spinal pain, alopecia, and myalgia), one was moderate and an SAE (ankle fracture), and one was severe and an SAE (lower limb fracture). The TEAE of alopecia was assessed as related to the study treatment.

Oro-dental:

- ENZ215: Two of the TEAEs were mild and non-serious (tooth disorder and skin mass [lower lip nodule left inner surface]) and three were moderate and non-serious (toothache, aphthous ulcer, and pain in jaw). The TEAEs of aphthous ulcer and pain in jaw were assessed as related to study treatment. Of

note, one additional participant had an AE that was not treatment-emergent of moderate periodontal inflammation.

Skin:

- ENZ215: Two of the TEAEs were mild and non-serious (skin disorder and food allergy), two were moderate and non-serious (skin infection and hypersensitivity), and one was moderate and serious (SAPHO syndrome). The TEAE of skin infection was assessed as related to study treatment.
- Prolia: One participant experienced a mild non-serious TEAE of arthropod sting that was assessed as not related to study treatment and resolved.

Gastrointestinal tract:

- Prolia: Two participants experienced moderate non-serious TEAEs of abdominal pain that were assessed as not related to study treatment; both cases resolved.

Cardiovascular system:

- ENZ215: One participant experienced a mild non-serious TEAE of arrhythmia supraventricular that was assessed as not related to study treatment.
- Prolia: One participant experienced a moderate non-serious TEAE of cardiac failure chronic, a severe SAE of myocardial infarction, and a mild non-serious TEAE of hypercholesterolemia. None of these TEAEs were assessed as related to study treatment.

Nervous system:

- ENZ215: One participant experienced a moderate non-serious TEAE of carpal tunnel syndrome that was assessed as not related to study treatment and resolved.

In the open-label extension period, one participant experienced a mild non-serious Oro-dental TEAE of tooth fracture that was assessed as not related to study treatment and resolved.

Other Observations Related to Safety

Double-blind treatment period:

All participants in the ITT set had a lateral lumbar X-ray performed at screening. Results showed that most participants (149 participants [58.9%] in the ENZ215 group and 160 participants [63.7%] in the Prolia group) had normal results; no participants had abnormal, clinically significant results. At Month 12, a total of 139 participants (54.9%) in the ENZ215 group and 143 participants (57.0%) in the Prolia group had normal results. One participant (0.4%) in ENZ215 group had abnormal clinically significant X-ray findings at Month 12.

One participant in the ENZ215 group had a mild, non-serious, non-related TEAE of spinal compression fracture on Study Day 359 (08 Feb 2024). The outcome of the TEAE was reported as recovered. Of note, this patient had abnormal, not clinically significant lumbar spine X-ray findings at baseline. The participant completed the study on Study Day 379 (28 Feb 2024).

Open-label extension period:

Overall, 118 (98.3%) participants had a lateral lumbar spine X-ray performed at Visit 11. One (1.7%) participant from each group did not undergo lumbar spine X-ray performed at Visit 11. Majority of the participants (46 participants [76.7%] in the ENZ215 group and 30 participants [50%] in the Prolia group)

had normal results. Overall, 41 (34.2%) participants (13 participants [21.7%] in the ENZ215 group and 28 participants [46.7%] in the Prolia group) had abnormal, not clinically significant X-ray findings. One participant (1.7%) in Prolia group had abnormal clinically significant X-ray findings.

One participant in the Prolia group had a mild, non-serious, non-related TEAE of lumbar vertebral fracture on Study Day 540 (10 Jun 2024). The event was ongoing at the time of report. Of note, this patient had abnormal, not clinically significant lumbar spine X-ray findings (fracture of vertebrae L3) at baseline. The participant completed the study on Day 540 (10 Jun 2024).

2.5.8.7. Immunological events

Bioanalytical methods

ECLIA based Method for Immunogenicity Assessment of Denosumab in Human Serum Samples

The Applicant has adopted an electrochemiluminescence immunoassay (ECLIA) bridging assay to screen, confirm and quantify denosumab specific antibodies in human serum matrix. The adopted three-tiered approach for determination of ADAs was well described and developed The presented method can be considered as valid.

Further, the Applicant presented an electrochemiluminescence (ECL) assay for detection of anti-denosumab neutralizing antibodies in human serum. The presented assay was well described and validated.

The impact of immunogenicity on PK and PD parameters were assessed for Enzene Denosumab and Prolia in both studies (ALK22/ENZ215-DEN1 and ALK22/ENZ215-DEN2).

In both studies, the immunogenicity testing strategy comprised of 3 stages:

- a screening stage, for the identification of potential ADA-positive samples,
- a confirmatory stage: for ensuring the accurate identification of true ADA-positive cases,
- a titration phase: for determination of ADA titres and NABs.

Anti-denosumab antibodies were detected using a validated screening assay. Enzyme-linked immune sorbent assay (ELISA) methods were used for screening assay, specificity assay and titre assay, to screen, confirm and determine the titres of denosumab ADAs. An ELISA method was also used to determine nABs against denosumab. Count and percentage were presented for immunogenicity endpoints.

Study ALK22/ENZ215-DEN1 (Healthy Adult Males)

All subjects had ≥1 post-treatment confirmatory ADA results across all treatment groups (ENZ215 [68 subjects, 100%], EU-sourced Prolia [69 subjects, 100%], US-sourced Prolia [69 subjects, 100%]); 1 subject each in ENZ215 (1.5%) and EU-sourced Prolia (1.4%) had confirmatory ADA, prior to study treatment administration.

From Day 16 onwards, the proportion of subjects with confirmatory ADA was comparable across all groups, with 94.1% in ENZ215 and 100% in both EU-Prolia and US- Prolia groups. This similarity in ADA development persisted through Days 28, 90, 180, and 270. The higher incidence of ADA could be due to higher sensitivity of assay methods (6.44 ng/mL) compared to that of the reference trials. There were eight patients (4 in ENZ5215 and EU-Prolia groups each) with relatively high ADA titer (>900), which appeared to be outliers.

Typically, titers in this study ranged between 20 and 700. For these eight patients, nAb results were negative on specified days, there was no pattern for gradual titer increase and none of these patients reported immune-related AEs, ISRs, or hypersensitivity. There was no consistent trend in PK and PD when comparing the results from 8 outliers with moderately high ADA titer (> 900) with the results from the rest of patients with ADA positive results.

Furthermore, there was no notable impact of ADA presence on the PK and safety for the patients who had ADA positive results. Please refer to integrated summary of immunogenicity for detailed results.

Table 49. Summary of Subjects Positive for ADA or NAb (Safety Analysis Set)

Parameters/Visit /Statistics	ENZ215 (N=68)		EU sourced Prolia® (N=69)	ENZ215 (N=68)		US sourced Prolia® (N=69)	All Subjects (N=206)
Parameter: ADA Confirmatory							
Baseline							
n (%)	1 (1.5)		1 (1.4)	1 (1.5)		0 (0.0)	2 (1.0)
Risk Difference [95% CI]		0.00 (-0.04,0.04)			0.01 (-0.01,0.04)		
p-value		1.0000			0.4964		
Day 8							
n (%)	46 (67.6)		52 (75.4)	46 (67.6)		39 (56.5)	137 (66.5)
Risk Difference [95% CI]		-0.08 (-0.23,0.07)			0.11 (-0.05,0.27)		
p-value		0.3170			0.1797		
Day 16							
n (%)	64 (94.1)		69 (100)	64 (94.1)		69 (100)	202 (98.1)
Risk Difference [95% CI]		-0.06 (-0.11, -0.00)			-0.06 (-0.11, -0.00)		
p-value		0.0580			0.0580		
Day 28							
n (%)	67 (98.5)		69 (100)	67 (98.5)		69 (100)	205 (99.5)
Risk Difference [95% CI]		-0.01 (-0.04,0.01)			-0.01 (-0.04,0.01)		
p-value		0.4964			0.4964		
Day 63							
n (%)	67 (98.5)		67 (97.1)	67 (98.5)		67 (97.1)	201 (97.6)
Risk Difference [95% CI]		0.01 (-0.03,0.06)			0.01 (-0.03,0.06)		
p-value		1.0000			1.0000		
Day 90							
n (%)	65 (95.6)		68 (98.6)	65 (95.6)		66 (95.7)	199 (96.6)
Risk Difference [95% CI]		-0.03 (-0.09,0.03)			-0.00 (-0.07,0.07)		
p-value		0.3658			1.0000		
Day 119							

n (%)	66 (97.1)		68 (98.6)	66 (97.1)		65 (94.2)	199 (96.6)
Risk Difference [95% CI]		-0.01 (-0.06,0.03)			0.03 (-0.04,0.10)		
p-value		0.6195			0.6805		
Day 147							
n (%)	65 (95.6)		65 (94.2)	65 (95.6)		64 (92.8)	194 (94.2)
Risk Difference [95% CI]		0.01 (-0.06,0.09)			0.03 (-0.05,0.11)		
p-value		1.0000			0.7183		
Day 180							
n (%)	52 (76.5)		49 (71.0)	52 (76.5)		55 (79.7)	156 (75.7)
Risk Difference [95% CI]		0.05 (-0.09,0.20)			-0.03 (-0.17,0.11)		
p-value		0.4682			0.6466		
Day 270/EOS							
n (%)	7 (10.3)		3 (4.3)	7 (10.3)		3 (4.3)	13 (6.3)
Risk Difference [95% CI]		0.06 (-0.03,0.15)			0.06 (-0.03,0.15)		
p-value		0.2073			0.2073		
Parameter: ADA_NAb							
Baseline							
n (%)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)
Risk Difference [95% CI]		NE (NE)			NE (NE)		
p-value		NE			NE		
Day 8							
n (%)	0 (0.0)		1 (1.4)	0 (0.0)		0 (0.0)	1 (0.5)
Risk Difference [95% CI]		-0.01 (-0.04,0.01)			NE (NE)		
p-value		1.0000			NE		
Day 16							
n (%)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)
Risk Difference [95% CI]		NE (NE)			NE (NE)		
p-value		NE			NE		
Day 28							
n (%)	0 (0.0)		1 (1.4)	0 (0.0)		1 (1.4)	2 (1.0)
Risk Difference [95% CI]		-0.01 (-0.04,0.01)			-0.01 (-0.04,0.01)		
p-value		1.0000			1.0000		
Day 63							
n (%)	1 (1.5)		1 (1.4)	1 (1.5)		0 (0.0)	2 (1.0)
Risk Difference [95% CI]		0.00 (-0.04,0.04)			0.01 (-0.01,0.04)		
p-value		1.0000			0.4964		

Day 90							
n (%)	2 (2.9)		1 (1.4)	2 (2.9)		1 (1.4)	4 (1.9)
Risk Difference [95% CI]		0.01 (-0.03,0.06)			0.01 (-0.03,0.06)		
p-value		0.6195			0.6195		
Day 119							
n (%)	0 (0.0)		0 (0.0)	0 (0.0)		1 (1.4)	1 (0.5)
Risk Difference [95% CI]		NE (NE)			-0.01 (-0.04,0.01)		
p-value		NE			1.0000		
Day 147							
n (%)	1 (1.5)		0 (0.0)	1 (1.5)		0 (0.0)	1 (0.5)
Risk Difference [95% CI]		0.01 (-0.01,0.04)			0.01 (-0.01,0.04)		
p-value		0.4964			0.4964		
Day 180							
n (%)	0 (0.0)		1 (1.4)	0 (0.0)		0 (0.0)	1 (0.5)
Risk Difference [95% CI]		-0.01 (-0.04,0.01)			NE (NE)		
p-value		1.0000			NE		
Day 270/EOS							
n (%)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)
Risk Difference [95% CI]		NE (NE)			NE (NE)		
p-value		NE			NE		

N = number of subjects; n = number of available subjects; NE = Not evaluable; 95% CI and p-value are calculated using difference in risk between the treatment's groups are compared using 2-sided Chi-Square or Fisher's exact test.
The sensitivity of ADA is 6.44 ng/mL.
Source: Table 14.2.3

Table 50. Post-hoc Analysis: Cumulative Summary of Subjects Positive for ADA or NAb (Safety Analysis Set)

Parameters/Visit /Statistics	ENZ215 (N=68)		EU sourced Prolia® (N=69)	ENZ215 (N=68)		US sourced Prolia® (N=69)	All Subjects (N=206)
Parameter: ADA Confirmatory							
Baseline							
n (%)	1 (1.5)		1 (1.4)	1 (1.5)		0 (0.0)	2 (1.0)
Risk Difference [95% CI]		0.00 (-0.04,0.04)			0.01 (-0.01,0.04)		
p-value		1.0000			0.4964		
Post Baseline							
n (%)	68 (100)		69 (100)	68 (100)		69 (100)	206 (100)
Risk Difference [95% CI]		NE (NE)			NE (NE)		
p-value		NE			NE		
Parameter: ADA_NAb							
Baseline							
n (%)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)
Risk Difference [95% CI]		NE (NE)			NE (NE)		
p-value		NE			NE		
Post Baseline							
n (%)	3 (4.4)		3 (4.3)	3 (4.4)		2 (2.9)	8 (3.9)
Risk Difference [95% CI]		0.00 (-0.07,0.07)			0.02 (-0.05,0.08)		
p-value		1.0000			0.6805		

Abbreviations: ADA = anti-Denosumab antibody; CI = confidence interval; N = number of subjects; n = number of cumulative subjects; NAb = neutralizing antibody; NE = Not evaluable.
Note(s): 95% CI and p-value are calculated using difference in risk between the treatment's groups are compared using 2-sided Chi-Square or Fisher's exact test.
Source: Table 14.2.3A

There were eight patients (4 in ENZ5215 and EU-Prolia groups each) with relatively high ADA titer (>900), which appeared to be outliers. Typically, titers in this study ranged between 20 and 700. Details for these outliers are as follows:

- Subject 03-001 in ENZ215 group had a titer value of 992 on Day 63 and 1210 on day 90;
- Subject 03-018 in ENZ215 group had a titer value of 920 on Day 63;
- Subject 03-054 in ENZ15 group had a titer value of 1040 on Day 63;
- Subject 03-056 in ENZ215 group had a titer value of 1110 on Days 147 and 180, and 1070 on Day 119.
- Subject 01-091 in EU-Prolia group had a titer value of 1280 on Day 63, and 1010 on Day 90;
- Subject 03-021 in EU-Prolia group had a titer value of 1060 on Day 90 and 1080 on Day 119;
- Subject 03-042 in EU-Prolia group had a titer value of 1070 on Day 28, 912 on Day 63 and 1020 on Day 119;
- Subject 03-064 in EU-Prolia group had a titer value of 1110 on Day 28.

For these eight patients, nAb results were negative on specified days, there was no pattern for gradual titer increase and none of these patients reported immune-related AEs, injection site reactions (ISRs), or hypersensitivity. There was no consistent trend in PK and efficacy when comparing the results from these 8 outliers with moderately high ADA titer (> 900) with the results from the rest of subjects with ADA positive results.

Impact of ADA on Individual patients PK

Table 51. PK parameters of subjects with high titer results (Study ALK22/ENZ215-DEN1)

Subject no.	C_{max} (ng/mL)	$AUC_{(0\text{-}inf)}$ (h*ng/mL)	$AUC_{(0\text{-}t)}$ (h*ng/mL)
ENZ215 treatment group			
03-001	6790.0	7435606.3	7230870.3
03-018	7580.0	6777650.7	6448021.1
03-054	11900.0	9533473.1	9473497.3
03-056	1760.0	1590552.7	1565099.4
EU-Prolia treatment group			
01-091	4420.0	7428012.2	7418659.0
03-021	8720.0	9890516.9	9589793.0
03-042	6260.0	6691185.1	6478921.2
03-064	9690.0	6883779.8	6742483.7
Mean results of all patients			
ENZ215 (N=68)	7995.29	8060737.96	7950718.07
EU-Prolia (N=69)	7930.87	8008973.81	7904876.44
US-Prolia (N=69)	8267.83	8248514.90	8151301.43

Source: CSR ALK22/ENZ215-DEN1 Listing 16.2.6.6. and CSR ALK22/ENZ215-DEN1, Table 5
Abbreviations: EU = European Union; US = United States

Study ALK22/ENZ215-DEN2 (post-menopausal osteoporosis)

In this clinical study, the immunogenicity profile of ENZ215 was evaluated in comparison to Prolia. ADA incidences and titers were assessed at multiple timepoints throughout the double-blind treatment period and open-label extension phase.

Table 52. Summary Statistics for Overall Immunogenicity Data

Visit/Day	Assay Type	Statistics	ENZ215 n (%)	Prolia® n (%)	Overall n (%)
Double-blind Treatment period			**N = 253**	**N = 251**	**N = 504**
Overall	ADA Confirmatory	Positive	252 (99.6)	247 (98.4)	499 (99.0)
		Negative	0	0	0
	NAb Status	Positive	1 (0.4)	5 (2.0)	6 (1.2)
		Negative	251 (99.2)	242 (96.4)	493 (97.8)
Overall - Postbaseline	ADA Confirmatory	Positive	252 (99.6)	247 (98.4)	499 (99.0)
		Negative	0	0	0
	NAb Status	Positive	1 (0.4)	5 (2.0)	6 (1.2)
		Negative	251 (99.2)	242 (96.4)	493 (97.8)
Open-label Extension period			**N = 60**	**N = 60**	**N = 120**
Overall	ADA Confirmatory	Positive	60 (100)	60 (100)	120 (100)
		Negative	0	0	0
	NAb Status	Positive	0	0	0
		Negative	60 (100)	60 (100)	120 (100)

N = number of patients in safety set; n = Number of patients with non-missing data within the specific category; n' = number of patients for whom titer result is available; n = Neutralizing antibody

Titer values of "Titer < 20" are considered as zero for the summary analysis.

Cut point analysis is considered for 112 subjects immunogenicity blood samples at baseline visit (Visit 2) and hence direct confirmatory tests are performed for these samples. In this way, all the subjects in the safety set got ADA testing done at Visit 2 (Day 1).

Percentages are calculated for Visit 2, Overall and Overall - Postbaseline based on N in the safety analysis set within the treatment group as the denominator whereas for the post-baseline visits, the percentages are calculated based on the number of samples available in ADA Screening assay at the visit in that treatment group as the denominator.

Source: Table 14.3.8.1 and Table 14.3.8.1a.

Double-blind treatment period:

At Visit 2 (Day 1), few participants had positive ADA confirmatory analysis (three [1.2%] and five [2.0%], respectively). For the eight participants with positive ADA confirmatory analysis at Visit 2, the mean (SD) ADA titers were 132.90 (147.279) in the ENZ215 group and 150.02 (61.957) in the Prolia group. None of these eight participants tested positive for NAbs. At Visit 5 (Day 30), 235 (93.6%) samples in the ENZ215 group and 228 (92.3%) samples in the Prolia group had had positive ADA confirmatory analysis. At Visit 6 (Day 90), nearly all samples had positive ADA confirmatory analysis (99.2% in both groups). The mean (SD) ADA titers were increased compared with Visit 2 (Day 1) at Visit 6 (Day 90) (190.32 [77.120] in the ENZ215 group and 168.09 [74.007] in the Prolia group) and at Visit 8 (Day 270) (194.28 [106.515] and 173.69 [80.735], respectively). At Visit 9 (Day 360) the mean (SD) ADA titers were below Visit 2 titer (91.82 [62.385] in the ENZ215 group and 83.22 [59.843] in the Prolia group). Post-baseline, overall all the 499 participants were positive in ADA confirmatory analysis. Of the 499 participants with positive ADA confirmatory analysis across all visits, one (0.4%) participant in the ENZ215 group and five (2.0%) participants in the Prolia group were positive for NAbs: one at Visit 5 (in the Prolia group), two at Visit 7 (both in the Prolia group) and three at Visit 8 (one in the ENZ215 group and two in the Prolia group). The higher ADA positivity rate was found as compared to data reported in SmPC/PI i.e. <1%. This might be result of the high sensitive ADA assay method (6.44 ng/mL) used in the current study.

The mean and the median values of ADA titer were not significantly different between the treatment groups at each timepoint, and the ADA titer values were relatively low during the overall study period. While most ADA positive patients had titer results between 100 and 400, there were 4 patients (all 4 patients in double-blind treatment period ENZ215 treatment group]) who had ADA titer level of >400. Details for these outliers are as follows:

- Patient 10030014 had a titer value of 496 on Day 270;
- Patient 10040005 had a titer value of 677 on Day 270;
- Patient 60061013 had a titer value of 584 on Day 270;
- Patient 60181039 had a titer value of 1670 on Day 180 and titer value of 997 on Day 270.

NAb results for all 4 patients were negative throughout the study and serum concentration levels at the time of high titer value observed were similar to the mean serum concentrations of either treatment groups. Comparison of area under the time effect curve (AUEC) of sCTX, percentage change in sP1NP and percent change from baseline in bone mineral density (BMD) showed no relevant effect of high titer development on these PD and efficacy endpoints in both treatment groups. No adverse event of related, serious, or Grade over 3 were reported for these 4 patients at any time during the study.

Table 53. Frequency of ADA and NAb during double-blind treatment period up to 12 Month in Study ALK22/ENZ215-DEN2: Safety Set

Visit	ENZ215 (N=253)	EU-Prolia (N=251)
	n (%)	
Day 1		
ADA Positive	3 (1.2)	5 (2.0)
NAb Positive	0	0
Day 30		
ADA Positive	235 (92.9)	228 (90.8)
NAb Positive	0	1 (0.4)
Day 90		
ADA Positive	246 (97.2)	245 (97.6)
NAb Positive	0	0
Day 180		
ADA Positive	212 (83.8)	209 (83.3)
NAb Positive	0	2 (0.8)
Day 270		
ADA Positive	234 (92.5)	237 (94.4)
NAb Positive	1 (0.4)	2 (0.8)
Day 360		
ADA Positive	218 (86.2)	202 (80.5)
NAb Positive	0	0

Source: CSR ALK22/ENZ215-DEN2 Table 14.3.8.1
Abbreviations: ADA = anti-drug antibody; EU = European Union; N = total number of patients; n = number of patients with the event; Nab = neutralizing antibody

Table: Summary of ADA Titer Results during double-blind treatment period up to Month 12 in Study ALK22/ENZ215-DEN2: Safety Set

Visit	Statistics	ENZ215 (N=253)	EU-Prolia (N=251)
Day 1	n	3	5
	Mean (SD)	132.90 (147.279)	150.02 (61.957)
	Median (Min, Max)	64.00 (32.7, 302.0)	166.0 (74.1, 230.0)
Day 30	n	235	228
	Mean (SD)	80.24 (80.732)	78.86 (84.871)
	Median (Min, Max)	70.10 (0.0, 320.0)	57.50 (0.0, 320.0)
Day 90	n	246	245
	Mean (SD)	190.32 (77.120)	168.09 (74.007)
	Median (Min, Max)	175.00 (35.9, 320.0)	151.00 (0.0, 320.0)
Day 180	n	212	209
	Mean (SD)	102.65 (126.721)	83.21 (70.979)
	Median (Min, Max)	79.70 (0.0, 1670.0)	69.90 (0.0, 320.0)
Day 270	n	234	237
	Mean (SD)	194.28 (106.515)	173.69 (80.735)
	Median (Min, Max)	172.00 (0.0, 997.0)	156.00 (0.0, 320.0)
Day 360	n	218	202
	Mean (SD)	91.82 (62.385)	83.22 (59.843)
	Median (Min, Max)	78.40 (0.0, 320.0)	73.10 (0.0, 320.0)

Source: CSR ALK22/ENZ215-DEN2 Table 14.3.8.1
Titer values of "Titer < 20" are considered as zero for the summary analysis.
Abbreviations: EU = European Union; Max = maximum; Min = minimum; N = number of patients in each treatment group; n =number of patients with the event; SD = Standard deviation.

Open-label extension period:

All samples in both the ENZ215 and Prolia groups were positive at ADA confirmatory analysis at Visit 10 (Day 450). The mean (SD) ADA titers at Visit 10 were comparable between the ENZ215 and Prolia groups, with values of 157.65 (72.635) and 146.12 (67.381), respectively. At Visit 11, a slight decrease in ADA reactivity was observed, with 55 (91.7%) samples in the ENZ215 group and 50 (83.3%) samples in the Prolia group having positive confirmatory analysis. The mean (SD) ADA titers at Visit 11 also showed a decrease compared to Visit 10 (87.70 [63.607] vs 81.86 [44.303]). No NAbs were detected in either treatment group throughout the open-label extension period.

Table 54. Frequency of ADA and NAb during open-label extension period up to 18 Months in Study ALK22/ENZ215-DEN2: Safety Set

Visit	ENZ215 (N=60)	EU-Prolia (N=60)
	n (%)	
Day 450		
ADA Positive	60 (100.0)	60 (100.0)
NAb Positive	0	0
Day 540		
ADA Positive	55 (91.7)	50 (83.3)
NAb Positive	0	0

Source: CSR ALK22/ENZ215-DEN2 Table 14.3.8.1a
Abbreviations: ADA = anti-drug antibody; EU = European Union; N = total number of patients; n = number of patients with the event; Nab = neutralizing antibody

Table 55. Summary of ADA Titer Results during double-blind treatment period up to Month 12 in Study ALK22/ENZ215-DEN2: Safety Set

Visit	Statistics	ENZ215 (N=60)	EU-Prolia (N=60)
Day 450	n	60	60
	Mean (SD)	157.65 (72.635)	146.12 (67.381)
	Median (Min, Max)	144.00 (0.0, 317.0)	127.50 (30.3, 320.0)
Day 540	n	55	50
	Mean (SD)	87.70 (63.607)	81.86 (44.303)
	Median (Min, Max)	74.00 (0.0, 320.0)	78.65 (0.0, 199.0)

Source: CSR ALK22/ENZ215-DEN2 Table 14.3.8.1a
Titer values of "Titer < 20" are considered as zero for the summary analysis.
Abbreviations: EU = European Union; Max = maximum; Min = minimum; N = number of patients in each treatment group; n =number of patients with the event; SD = Standard deviation.

2.5.8.8. Discontinuation due to adverse events

2.5.8.8.1. Healthy male population (Study ALK22/ENZ215-DEN1)

Discontinuations Due to Adverse Events

No TEAE leading to discontinuation of study treatment was reported.

2.5.8.8.2. Patients with postmenopausal osteoporosis (Study ALK22/ENZ215-DEN2)

Double-blind treatment period:

One participant (0.4%) in the ENZ215 group and two participants (0.8%) in the Prolia group experienced TEAEs leading to treatment and study withdrawal.

In the ENZ215 group, the event concerned a severe, non-related, TESAE of pneumonia.

In the Prolia group, the events concerned a TESAE of breast cancer and a non-serious TEAE of rash. Both TEAEs were moderate in severity and not related to study treatment.

Three participants in the ENZ215 group experienced four TEAEs leading to treatment interruption. These included the following (occurring in one participant each): pain in jaw (moderate severity, non-serious, assessed as related to study treatment); large intestine polyp (mild severity, non-serious, assessed as not related to study treatment); adenocarcinoma of colon (same participant that experienced large intestine polyp) (moderate severity, serious, assessed as not related to study treatment); and hypocalcaemia (mild severity, non-serious, assessed as related to study treatment).

Two participants in the Prolia group experienced two TEAEs leading to treatment interruption: ankle fracture (moderate severity, serious, assessed as not related to study treatment) and lung neoplasm (moderate severity, non-serious, assessed as not related to study treatment).

Open-label extension period:

No discontinuation of study treatment or study withdrawal due to TEAEs were reported during the open-label extension period.

None of the TEAEs led to treatment interruption during the open-label extension period.

2.5.8.9. Post marketing experience

Not applicable

2.5.9. Discussion on clinical safety

Comparability of safety and immunogenicity between the biosimilar candidate ENZ215 and the reference medicinal product Prolia was investigated in two pivotal studies with different patient populations and duration of treatment:

- ALK22/ENZ215-DEN1, a Phase 1, randomized (1:1:1), double-blind, three-arm, parallel-group, single-dose study to compare the pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity of ENZ215, EU-Prolia, and US-Prolia in healthy adult male volunteers for a period of 270 days.
- ALK22/ENZ215-DEN2, a Phase 3, randomized (1:1), double-blind, parallel-group, active-controlled study to compare the efficacy, safety, PD, PK and immunogenicity of ENZ215 with EU-Prolia in postmenopausal women with osteoporosis (PMO) for a period of 12 months (360 days). Patients initially randomized to EU-Prolia and completed the double-blind period were re-randomized 1:1 to either receive ENZ215 or EU-Prolia for an additional 6 months and 1 dose.

The design of both studies is considered acceptable for assessment of comparability between the respective treatments.

While the Applicant applied severity grading of an event in study ALK22/ENZ215-DEN1 according to the latest version of CTCAE (Common Terminology Criteria for Adverse events), as per the latest study protocol

(Version 4.0), this was not done for study ALK22/ENZ215-DEN2 (as per the latest study protocol (Version 3.0)). Thus, the Applicant was asked why CTCAE was not used for severity grading in the Phase 3 study ALK22/ENZ215-DEN2. In their response, the Applicant noted that this difference was due to the standard operating procedures (SOPs) of the respective Contract Research Organizations (CROs) involved in protocol development. Since the protocol-noted definitions for mild, moderate, and severe for assessment of AE intensity/severity did not raise particular concerns and a negative effect that would jeopardize study's ALK22/ENZ215-DEN2 data or interpretability thereof is not presumed (as the primary period of the study was double-blinded with a parallel-group design and an active comparator), no further concern remains in this regard.

The safety (analysis) set of each study consisted of all subjects who received at least one dose of the study drug. One year double-blinded safety comparability data between ENZ215 and EU-Prolia are available from the Phase 3 study ALK22/ENZ215-DEN2 with PMO patients. It is noted that the CHMP does not require data on interchangeability (switch between treatments), thus, one-year data is sufficient. Overall, the number of subjects and extent of exposure in the respective safety sets is deemed adequate to allow the assessment of comparable safety and immunogenicity of ENZ215 with Prolia.

Adverse events

In the Phase 1 study with healthy male subjects, most TEAEs were reported to be mild (83 [40.3%]) or moderate (50 [24.3%] subjects); severe TEAEs were only reported in 2 (1.0%) subjects. Incidence of total TEAEs was slightly higher in the EU-Prolia group than in the ENZ215 and US-Prolia groups, with 39 (56.5%), 28 (41.2%), and 32 (46.4%) of the subjects, respectively. This trend is also noticeable when comparing incidence of mild and moderate TEAEs, with 25 (36.8%), 25 (36.2%), and 33 (47.8%) for mild TEAEs in ENZ215, US-Prolia, and EU-Prolia groups, and 12 (17.6%), 18 (26.1%), and 20 (29.0%) for moderate TEAEs in ENZ215, US-Prolia, and EU-Prolia groups, respectively. However, this trend was not observed in the incidence of TEAEs related to the study drug by investigator judgement, which were reported in 5 (7.4%), 4 (5.8%), and 6 (8.7%) subjects, in the ENZ215, US-Prolia, and EU-Prolia groups, respectively. Moderate to severe TEAEs were more common in the EU-Prolia group (21 subjects, 30.4%) and US-Prolia group (18 subjects, 26.1%) compared to ENZ215 (13 subjects, 19.1%).

Overall, the reported incidence of TEAEs by SOC and PT in healthy subjects was similar in the ENZ215, US-Prolia, and EU-Prolia groups, with SOCs 'infections and infestations' and 'injury, poisoning and procedural complications', and PTs 'nasopharyngitis' and 'upper respiratory tract infections' being the exception: The incidence of SOC 'infections and infestations' was reported in 13 (19.1%), 17 (24.6%), and 20 (29.0%) subjects, and SOC 'injury, poisoning and procedural complications' with 3 (4.4%), 0, and 7 (10.1%) subjects, in the ENZ215, US-Prolia, and EU-Prolia groups, respectively. The incidence of TEAEs for PT 'nasopharyngitis' was reported in 6 (8.8%), 13 (18.8%), and 11 (15.9%) subjects, in the ENZ215, US-Prolia, and EU-Prolia groups, and for PT 'upper respiratory tract infection' in 3 (4.4%), 1 (1.4%), and 7 (10.1%) subjects, in the ENZ215, US-Prolia, and EU-Prolia groups, respectively. Although TEAE incidence of SOCs 'infections and infestations' and 'injury, poisoning and procedural complications' and of PT 'nasopharyngitis' was lower in the ENZ215 group compared to the Prolia groups, the incidence of TEAEs for other SOCs or PTs (including also PT 'upper respiratory tract infection') did not corroborate this observation. Of the TEAEs with an incidence of >5%, nasopharyngitis was reported in 8.8% of patients in the ENZ215 group compared to 18.8% in US-Prolia group and 15.9% in EU-Prolia group, and headache was reported in 13.2% of patients in the ENZ215 group, 20.3% in the US-Prolia group and 13.0% in the EU-Prolia group.

In the double-blinded phase of the Phase 3 study with PMO patients, most TEAEs were reported to be mild or moderate in severity. One SUSAR (suspected unexpected serious adverse reaction) and one fatal (i.e.,

leading to death) AE/TEAE were reported, both in the Prolia group. Seven TEAEs graded severe were reported in the ENZ215 group in 5 patients (2.0%), whereas five were reported in the Prolia group in 5 patients (2.0%). In detail, those were influenza, pneumonia, staphylococcal bacteraemia, myasthenia gravis, radius fracture, ankle fracture, and genital prolapse in the ENZ215 group and COVID-19, pneumonia, lower limb fracture, myocardial infarction, and chromophobe renal cell carcinoma in the Prolia group; none of these were considered related to study treatment by the investigator, which is agreed to.

The number of TEAEs among PMO patients was higher in the ENZ215 group than in the Prolia group (441 events vs. 393 events) but affected a similar number and percentage of patients as those were reported in 164 (64.8%) patients of the ENZ215 group and in 157 (62.5%) patients of Prolia group. Additionally, no significant differences were observed when comparing incidence of severe TEAEs (as noted above), and TEAEs assessed related to study treatment by the investigator (ENZ215: 25 events reported in 19 [7.5%] patients, Prolia: 36 events reported in 23 [9.2%] patients, respectively). The most frequent reported TEAEs by SOC were in SOC 'infections and infestations' (251 events in 172 (34.1%) patients, followed by SOC 'musculoskeletal and connective tissue disorders' (108 events in 79 (15.7%) patients. As regards the most frequently reported TEAEs by PT, these were 'nasopharyngitis' (45 events in 40 [7.9%] patients) and 'upper respiratory tract infection' (32 events in 27 [5.4%] patients). Of note, PTs 'pain in extremity' and 'musculoskeletal pain' within SOC 'musculoskeletal and connective tissue disorders', and PTs 'urinary tract infection' and 'common upper respiratory tract infection' within SOC 'infections and infestations' are listed as 'very common' (i.e., ≥ 1/10) and 'common' (i.e., ≥ 1/100 to < 1/10) as per Prolia SmPC section 4.8. Fracture TEAEs were similar between treatment arms. A total of 16 fractures (4.0%) were reported in participants in the ENZ215 group and 8 fractures (2.8%) in the Prolia group. None of the fractures were considered related to study treatment. Overall, there were no significant differences as regards incidence of TEAEs by SOC and PT.

Initially, no detailed information (e.g., number and percentages of mild or moderate TEAEs, separately) was presented, hence, the Applicant was requested to provide that. With their responses, the Applicant provided those data: The incidence of mild TEAEs was similar between the two treatment arms with 244 events reported in 115 (45.5%) patients of the ENZ215 group and 249 events reported in 119 (47.4%) patients of the Prolia group. However, the incidence of moderate TEAEs differed between the two arms as 190 events were reported in 105 (41.5%) patients of the ENZ215 group versus 139 events were reported in 84 (33.5%) patients of the Prolia group. Nevertheless, since more detailed analyses i.e. incidence of TEAEs assessed related to study treatment by the investigator, and incidence by SOC or PT categorization (see above) showed no striking imbalance, no concerns remain as regards this matter.

In the open-label phase of the Phase 3 study with PMO patients, all of the TEAEs were mild or moderate in severity. There were no SUSAR, SAE, fatal AE, or AE leading to treatment withdrawal reported during the open-label extension period. Alike the double-blind phase, the number of TEAEs was higher in the ENZ215 group than in the Prolia group (36 events vs. 30 events) but affected a similar number and percentage of patients as those were reported in 23 (38.3%) patients of the ENZ215 group and in 23 (38.3%) patients of Prolia group. TEAEs considered related by the investigator were only reported in the ENZ215 group (3 events in 2 (3.3) patients), however, this relatively low number and percentage do not raise concerns. The most frequent reported TEAEs by SOC were in SOC 'infections and infestations' (28 events in 27 (22.5%) patients, followed by SOC 'musculoskeletal and connective tissue disorders' (8 events in 7 (5.8%) patients. Regarding the most frequently reported TEAEs by PT, these were 'nasopharyngitis' (11 events in 10 [8.3%] patients); all other TEAEs by PT were reported with either 1 or 2 events in the population. In the open-label extension period, 2 fracture TEAEs, not related to study treatment, were reported in 2 subjects (3.3%) in the Prolia

group. Similar to the double-blind phase, there were no concerning differences as regards the incidence of TEAEs by SOC and PT.

Initially, similar to the double-blinded phase, no detailed information (e.g., number and percentages of mild or moderate TEAEs, separately) was presented for the open-label phase. Hence, the Applicant was requested to provide that. With their responses, the Applicant provided those data. Although the number of mild TEAEs was higher in the ENZ215 group compared to the Prolia group (23 vs 13 events, respectively), the number and percentage of patients who experienced those events were the same (n=13, 21.7%). The number of moderate TEAEs was lower in the ENZ215 group compared to the Prolia group (13 vs 17 events) but these were reported in similar numbers and percentages between the treatment groups (11 [18.3%] patients of the ENZ215 group vs. 12 [20.0%] patients of the Prolia group). Taken together, these data do not indicate a reason for concern.

Considering both the results of the healthy male subjects and the PMO patients, no significant differences between ENZ215 and Prolia are concluded as regards adverse event and adverse drug reactions.

Serious adverse events

One healthy subject in the EU-Prolia group experienced a ligament rupture that resulted in hospitalization. This event was considered as not related to study treatment by the investigator.

In the double-blind phase of the Phase 3 study, all SAEs were treatment-emergent with 40 events in 31 (6.2%) of patients. Most of those (i.e., TESAEs) were in the SOC 'injury, poisoning and procedural complications' with 17 events in 14 (2.8%) patients, of which the majority were fractures. No significant differences were reported as regards the incidence of TESAEs (ENZ215: 23 events reported in 16 [6.3%], Prolia: 17 events reported in 15 [6.0%] patients, respectively). A TEAE of cystitis of a patient in the Prolia group (0.4%) was assessed as related by the investigator and thus considered a SUSAR.

No SAE or SUSAR was reported during the open-label extension period.

Deaths

There were no deaths in the healthy subject study. One death occurred in a patient in the Prolia group (that was not enrolled in the open label phase) on study day 385, caused by a TESEA of Covid-19. The investigator assessed that as not related to study treatment, to which it is agreed to.

Adverse events of special interest

For the Phase 1 study in healthy subjects, a mention or definition of adverse event of special interest (AESI) is totally absent from the CSR or study protocol. Notably, incidence of hypocalcemia, which is defined as AESI in the Phase 3 study with PMO patients and also in other denosumab procedures, due to being a potential risk with denosumab treatment (listed in 4.4 and 4.8 of the Prolia SmPC), was similar across the ENZ215, US-Prolia, and EU-Prolia groups, in 3 (4.4%), 2 (2.9%), and 3 (4.3%) of subjects, respectively. All these events were mild or moderate in intensity and were resolved.

For the Phase 3 study in PMO patients, AESIs were defined as hypocalcaemia, osteonecrosis of the jaw (ONJ), atypical femoral fracture, fracture healing complications, severe infection (including skin infection) not leading to hospitalisation, hypersensitivity leading to emergency room visit and potential Hy's law. The choice for these events is not explained by the Applicant. However, the Prolia SmPC lists 'hypocalcaemia', 'skin infections', 'osteonecrosis of the jaw (ONJ)', and 'atypical fractures of the femur' in sections 4.4 and 4.8, and 'drug-related hypersensitivity reactions' in section 4.8; hence, these are acceptable.

In the double-blind treatment period, all AESIs were treatment-emerging and were reported with similar incidence between the two groups with 8 events in 8 patients (3.2%) in the ENZ215 group and 7 events in 7 patients (2.8%) in the Prolia group, respectively. The majority of AESIs were hypocalcaemia with 7 events in 7 patients (2.8%) in ENZ215 group and 6 events in 6 patients (2.4%) in the Prolia group, respectively, all assessed as related to study drug except one in the Prolia group (a 65-year-old patient). For this patient, the Applicant had been asked to provide a detailed narrative of this event including a detailed, comprehensive and comprehensible explanation for the investigator's decision to assess said event as non-related to study drug. In their response, the Applicant stated that a low corrected calcium (2.1 mmol/L) was observed in the routine sample analysis at the Central Lab for that subject at Visit 4 (Day 15, double-blinded period) and that this was initially assessed by the PI as not clinical significant because the patient was asymptomatic and, therefore, no AE was reported. The Applicant further stated that however, since an action had been taken to manage this finding (increased dose of calcium supplements), the PI was instructed to report the applicable AE and that the Investigator assessed the event of hypocalcaemia to be not related to the study intervention. The Applicant further elaborated that the investigator was queried several times since it is an expected event after denosumab administration as per literature and that the PI confirmed the assessment of not related in all instances but did not provide a justification (last response was "*no medical sense to be related*")." The PI further explained that "*the result was considered accidental, with no reasonable explanation for it*", and after further questioning, where it was highlighted that low calcium in this patient had presented within two weeks +/- 3 days from IP administration, which is the timeframe when hypocalcemia in the context of denosumab treatment has been described, the PI indicated that "*having in mind this it might be considered probably related*". Although the handling of this event is not considered optimal, no further question is raised as it does not change the distribution of events. All events of hypocalcaemia were non-serious and mild in severity, except for one event that was moderate in severity, experienced by a 71-year-old patient in the ENZ215 group. Two other AESIs were reported: One was a TESAE of fracture displacement (moderate severity) experienced by a 71-year-old patient in the ENZ215 group, assessed as not related to study treatment by the investigator. For this event, the Applicant had been requested to explain the rationale for the non-relatedness decision. With their response, the Applicant provided information including narratives of two events experienced by that PMO patient (double-blinded phase, ENZ215 group). That patient experienced a SAE of radius fracture (fracture of the distal epiphysis of the left radial bone) and two weeks afterwards a SAE/AESI of fracture displacement (displacement of fracture of the distal epiphysis of the left radius bone). Both events were assessed as not related to study intervention by the investigator and the sponsor, which is agreed to. The other AESI was a non-serious TEAE of pneumonia (graded severe) in a 74-year-old patient in the Prolia group, assessed as not related to study treatment by the investigator. In this regard, the reasons for considering this event as AESI had been unclear and thus the Applicant had been asked to explain why this event was regarded as AESI whereas other events of pneumonia were not (despite e.g., being assessed as SAE). In their response, the Applicant explained that the rationale behind this approach was that in contrast to a severe infection requiring hospitalization which would be immediately reported to the sponsor as a SAE, a severe infection not leading to hospitalization may remain unnoticed. Hence, severe infections not leading to hospitalization were considered to be an AESI and thus require immediate reporting (i.e. within 24hrs) for safety monitoring purposes. This rationale can be understood and thus this concern is resolved. All the AESIs were recovered except for one AESI of hypocalcaemia in the ENZ215 group that was as ongoing and experienced by a 56-year-old patient on Day 364. No AESI was reported during the open-label extension period.

TEAE leading to discontinuation of study treatment

No TEAE leading to discontinuation of study treatment was reported in the healthy subject study.

In the double-blind treatment period of the PMO patient study, TEAEs leading to treatment and study withdrawal were rare and with similar incidence between the two groups: One patient (0.4%) in the ENZ215 group, which concerned an event of pneumonia (graded severe and not related to study treatment by the investigator, which is agreed to) and two patients (0.8%) in the Prolia group (TESAE of breast cancer and a TEAE of rash, both graded moderate and not related to study treatment by the investigator, which is agreed to). TEAEs leading to treatment interruption were also rare and reported with similar incidence between the two groups: Four TEAEs experienced by three patients in the ENZ215 group (pain in jaw, graded moderate, non-serious, and assessed as related to study treatment; large intestine polyp, graded mild severity, non-serious, and assessed as not related to study treatment; adenocarcinoma of colon, experienced by the same participant that experienced the large intestine polyp, graded moderate severity, serious, and assessed as not related to study treatment; and hypocalcaemia, graded mild, non-serious, and assessed as related to study treatment), and two TEAEs in two patients in the Prolia group (ankle fracture, graded moderate, serious, and assessed as not related to study treatment; and lung neoplasm, graded moderate, non-serious, and assessed as not related to study treatment). During the open-label extension period, no discontinuation of study treatment or study withdrawal due to TEAEs were reported and none of the TEAEs led to treatment interruption.

Laboratory and other findings

Concerning laboratory and other findings, significant differences indicating a particular trend were not reported and thus no concern is raised, for both studies.

Immunogenicity

The applied immunogenicity testing strategy comprised of 3 stages in both studies (screening for the identification of potential ADA-positive samples, confirmatory for ensuring the accurate identification of true ADA-positive cases, and titration for determination of ADA and NAbs) is deemed acceptable.

In the initially provided documentation, an integrated summary of immunogenicity had not been included, despite it being mentioned and referenced. Upon request, the Applicant provided that document.

The reported incidence of ADA-positive subjects was extremely high among the healthy study subjects and increased rapidly with 94.1% in ENZ215 and 100% in both EU-Prolia and US-Prolia groups as early as Day 16, however, declined after Day 147 until study end. The decline is expected as immune responses typically wane with time and this was a single-dose study. As regards the extremely high incidence (e.g., Day 28: 67 subjects (98.5%) in ENZ 215, 69 subjects (100%) in EU-Prolia, and 69 subjects (100%) in US-Prolia), this is likely a result of the high sensitivity of the applied assay (6.44 ng/mL cut-off). No significant differences in ADA incidence between the groups were reported. Baseline ADA were detected in only 2 patients in the Phase 1 study. Titers were very high in some subjects; in the Phase 1 study eight subjects had titers > 900.

Similarly to the healthy subjects, the incidence of ADA-positive PMO patients in the double-blind phase was extremely high (e.g., at the second ADA sampling at Visit 5 (Day 30), a positive ADA confirmatory analysis in 235 (93.6%) in the ENZ215 group and 228 (92.3%) in the Prolia group). The incidence was reported to be lower to some extent at Visit 7 (Day 180) and Visit 9 (Day 360), which is not unexpected as those were the visits with utmost timely distance to the last treatment administration. As aforementioned and also applicable for the PMO patient study, the high ADA incidence is likely a result of the high sensitivity of the applied assay. In the open-label extension phase, at Visit 10 (Day 450) all patients (i.e., in both groups) had confirmed ADA. ADA incidence declined as indicated by the Visit 11 (Day 540) results: 55 (91.7%) patients in the ENZ215 group and 50 (83.3%) patients in the Prolia group had confirmed ADA. Significant differences in ADA incidence between the groups were not observed for either phase. Baseline ADA were detected in only 8

patients in the Phase 3 study. In-depth titer data are not presented for the subjects in the Phase 3 study; mean titers, however, were presented and were 190 and 168, in the ENZ215 and Prolia group, respectively. These titers are considered high, i.e. ADA titers > 100-200 are generally considered to be of clinical relevance.

Incidence of NAbs in healthy subjects was extremely low compared to total ADA incidence, e.g., on Day 28: 1 subject (1.4%) each in EU-Prolia and US-Prolia groups and no subject in the ENZ215 group. On Day 270 no subject had NAb positive across treatment groups. No significant differences in NAb incidence between the groups were reported.

For PMO patients, like for the healthy subjects, incidence of NAbs was extremely low compared to total ADA incidence, e.g., only one subject in the Prolia group (0.4%) at Visit 5 (Day 30). Additionally, there were no significant differences in NAb incidence observable between the groups. Furthermore, no patients had NAbs in either treatment group throughout the open-label extension period.

Only one case of drug hypersensitivity was reported in both studies, experienced by a patient in the ENZ215 group of the open-label phase, however, no concern is raised considering this single event.

Taken together, as with all therapeutic proteins, there is a potential for denosumab for immunogenicity. In the two studies, a significantly higher incidence of total ADA in all groups was reported compared to ADA incidence reported in the studies for MAA of Prolia. As aforementioned, this might be a result of the high sensitivity of the applied assay (6.44 ng/mL cut-off). Considering the high incidence of ADAs, which is not in line with the Prolia EPAR and may be attributed to target interference of sRANKL or assay sensitivity (Don Zhong et. Al. 2017, DOI: 10.1208/s12248-017-0148-7). Upon request, the Applicant provided data and relevant discussion, from which it can be concluded that the PK parameters are largely comparable between ENZ215 and US Prolia across both ADA-negative and ADA-positive subgroups. In addition, for study ALK22/ENZ215-DEN2, the Applicant had been asked to present an analysis of the %CfB in the lumbar spine BMD (change from baseline on days 26 and 52) for ADA-positive subjects in groups of titers (quartiles) versus the ADA-negative subjects for both treatment groups. Based on these analyses provided, no clear trend was apparent, which indicates that there is no apparent effect of ADA titer level on efficacy.

2.5.10. Conclusions on the clinical safety

Overall, the collected safety and immunogenicity data are indicative of comparable safety and immunogenicity between the biosimilar candidate ENZ215 and the RMP Prolia.

2.6. Risk Management Plan

2.6.1. Safety concerns

Table 56. Summary of safety concerns

Summary of safety concerns	
Important identified risks	• Hypocalcemia • Skin infection leading to hospitalization • Osteonecrosis of the jaw • Hypersensitivity reactions • Atypical femoral fracture • Hypercalcemia in pediatric patients receiving denosumab and after treatment discontinuation
Important potential risks	• Fracture healing complications • Infection • Cardiovascular events • Malignancy
Missing information	• None

2.6.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.6.3. Risk minimisation measures

Table 57. Description of Routine Risk Minimisation Measures by Safety Concern

Safety Concern	**Routine Risk Minimisation Activities**
Important Identified Risks	
Hypocalcaemia	Routine risk communication: • SmPC Sections 4.2, 4.3, 4.4 and 4.8 • Package leaflet (PL) Sections 2 and 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • Recommendation for correction of hypocalcemia prior to initiating treatment with Osqay and clinical monitoring of calcium levels during treatment with Osqay is included in SmPC Section 4.4.
Skin infection leading to hospitalisation	Routine risk communication: • SmPC Sections 4.4 and 4.8

	• PL Sections 2 and 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Osteonecrosis of the jaw	Routine risk communication: • SmPC Sections 4.4 and 4.8 • PL Sections 2 and 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • Recommendation for oral examination, maintenance of good oral hygiene during treatment, management of patients with unavoidable invasive dental procedures, and temporary interruption of treatment if ONJ occurs is included in SmPC Section 4.4.
Hypersensitivity reactions	Routine risk communication: • SmPC Sections 4.3 and 4.8 • PL Sections 2 and 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Atypical femoral fracture	Routine risk communication: • SmPC Sections 4.4 and 4.8 • PL Sections 2 and 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • Recommendation for reporting new or unusual thigh, hip, or groin pain is included in SmPC Section 4.4.
Hypercalcemia in pediatric patients receiving denosumab and after treatment discontinuation	Routine risk communication: • SmPC Sections 4.2, 4.4 and 4.8 • PL Section 2 Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Important Potential Risks	

Fracture healing complications	Routine risk communication: • SmPC Section 5.3 Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Infection	Routine risk communication: • SmPC Section 4.8 • PL Section 4 Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Cardiovascular events	Routine risk communication: • None Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Malignancy	Routine risk communication: • None Routine risk minimization activities recommending specific clinical measures to address the risk: • None.
Missing Information	
None	N/A

ONJ=osteonecrosis of the jaw; PL=package leaflet; SmPC=summary of product characteristics.

Table 58. Additional Risk Minimization Measure: Patient Reminder Card

Objectives	Patient reminder cards will be provided to address the following risk (Annex 6): • Osteonecrosis of the jaw
Rationale for the additional risk minimization activity	The purpose of the patient reminder card is to remind patients about important safety information that they need to be aware of before and during treatment with denosumab (Osqay) injections for osteoporosis and bone loss, including: • the risk of osteonecrosis of the jaw during treatment with Osqay;

	• the need to highlight any problems with their mouth or teeth to their doctors/nurses before starting treatment; • the need to ensure good oral hygiene during treatment; • the need to inform their dentist of treatment with Osqay and to contact their doctor or dentist if problems with the mouth or teeth occur during treatment.
Target audience and planned distribution path	Target audience will be the patients. The patient reminder card will be distributed as per the approved dissemination plan agreed with each relevant national agency.
Plans to evaluate the effectiveness of the interventions and criteria for success	Monitor and evaluate postmarketing safety data and report in periodic safety update reports (PSURs). The distribution of the patient reminder card will be tracked to ensure that it is distributed in accordance with the dissemination plan agreed with each national agency. Additional requests for patient reminder cards and, where relevant web downloads, will also be recorded as an indicator of ongoing use of the patient reminder card. The effectiveness of risk minimization of ONJ in the EU will be monitored through postmarket reporting rates of ONJ after introduction of the patient reminder card.
Evaluation of the effectiveness of risk minimization activities	No change in risk-benefit profile

EU=European Union; ONJ=osteonecrosis of the jaw; PSUR=periodic safety update report.

2.6.4. Conclusion

The CHMP considers that the risk management plan is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

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

2.7.2. Periodic Safety Update Reports submission requirements

2.7.3. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.7.4. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Osqay (Denosumab) is included in the additional monitoring list as it is a biological product.

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

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

Osqay (ENZ215) was developed as a biosimilar product to Prolia (INN: denosumab), marketed by Amgen and was developed with the same strength and presentation (Prolia: 60 mg/mL PFS). Prolia is indicated for:

- Treatment of osteoporosis in postmenopausal women and in men at increased risk of fractures. In postmenopausal women Prolia significantly reduces the risk of vertebral, non-vertebral and hip fractures.
- Treatment of bone loss associated with hormone ablation in men with prostate cancer at increased risk of fractures. In men with prostate cancer receiving hormone ablation, Prolia significantly reduces the risk of vertebral fractures.
- Treatment of bone loss associated with long-term systemic glucocorticoid therapy in adult patients at increased risk of fracture.

For this MAA, the Applicant intends to claim all of the indications of the reference product.

Summary of Quality data

On a quality level the Applicant compared ENZ5121 with reference medicinal product Prolia by investigating descriptive quality attributes of the denosumab molecule with state-of-the-art methodology. Given the resolution of the concerns mentioned under 3.1.3.5 and in the list of questions in 3.2.R, ENZ5121 can be regarded biosimilar to Prolia.

An extensive summary on the analytical biosimilarity assessment is provided under 3.1.3.5.

Summary of Nonclinical data

The non-clinical comparative assessment included a battery of in vitro functional activity studies which are presented under the Quality section. In addition, two toxicity studies were conducted: a comparative 28-day

repeat-dose subcutaneous toxicity study in Sprague Dawley rats with a 14-day recovery period, assessing the systemic toxic potential of Enzene Denosumab (ENZ215) when administered as weekly subcutaneous injections compared to Prolia and a comparative 28-day repeat-dose subcutaneous toxicity study in New Zealand White rabbits with a 14-day recovery period, also assessing the systemic toxic potential of ENZ215 under the same conditions. These studies were shared in this application for information only.

Summary of Clinical data

ENZ215 is a biosimilar product of Prolia (denosumab). The clinical development program of ENZ215 included two clinical studies to demonstrate similarity in PK, PD, efficacy, safety and immunogenicity of ENZ215 and the reference product:

ALK22/ENZ215-DEN1 was a randomized, double-blind, three-arm, parallel-group, single-dose study to compare the PK, PD, safety, tolerability, and immunogenicity of Denosumab (ENZ215, EU-sourced Prolia, and US-sourced Prolia) in healthy adult male volunteers. The study was conducted at three investigational sites in three countries (Bulgaria, Poland, and UK). Subjects were randomised in a 1:1:1 ratio to one of the three treatments groups without stratification. The study had a duration of 270 days (EOS), plus a screening period of up to 28 days prior to IP administration on Day 1. Overall, the design of study ALK22/ENZ215-DEN1 is acceptable and is generally in agreement with previous Scientific Advice received from EMA.

ALK22/ENZ215-DEN2 was a Phase 3, randomized, double-blind, parallel-group, active-controlled study to compare the efficacy, safety, PD, PK and Immunogenicity of ENZ215 and EU-Prolia in postmenopausal women with osteoporosis. The study was conducted in 44 investigative sites that enrolled participants across the EU (Bulgaria, Czech Republic, Denmark, Lithuania, Poland, Serbia, Spain). The study was divided into three periods: Screening period: up to 35 days; Double-blind treatment period of 12 months; and Open-label, switch-over period of six months. Subjects were randomized in a 1:1 ratio for the main, double-blind treatment period. A subset of patients of the Prolia arm were offered to enrol in the open-label, switch-over extension period, where patients were re-randomized in a 1:1 ratio to receive either another dose of Prolia or switch to ENZ215. In total, patients received two s.c. denosumab doses during the double-blind period (on Day 1 and Month 6) and another dose during the open-label period (at Month 12). Overall, the design of study ALK22/ENZ215-DEN2 is acceptable and is generally in agreement with previous Scientific Advice received from EMA.

3.2. Results supporting biosimilarity

Quality

As mentioned in 3.1.3.5 and 3.1.4 ENZ5121 can be regarded biosimilar to Prolia due to the sum of results obtained from an extensive analytical comparability exercise.

Nonclinical

The supportive repeat-dose toxicity study in in Sprague Dawley rats and New Zealand White rabbits did not reveal significant differences between ENZ2151 and EU-Prolia in the toxicokinetic and toxicology endpoints. These data are provided by the Applicant for information purpose only as, by EMA-guidance, in vivo animal studies are not required for biosimilarity assessment.

Clinical

PK

DEN1 study (main PK study):

Biosimilarity in PK of ENZ215 and EU-Prolia (and US-Prolia) was shown in healthy male subjects. The ratio (ENZ215/EU-Prolia) of the geometric LS mean for C_{max} was 98.97% with the corresponding 90% CI being [91.52%, 107.03%]. The ratio of the geometric mean for AUC_{0-t} was 97.83% with the 90% CI being [89.60%, 106.81%]. The ratio of the geometric mean for AUC_{0-inf} was 97.98% with the corresponding 90% CI being [89.82%, 106.89%]. Thus, all primary PK endpoints were met as all results were within the pre-defined equivalence margin of (80% and 125%).

The secondary PK parameters (i.e., $pAUC_{0-D28}$, T_{max}, $T_{1/2}$, and CL/F) were comparable between the treatments supporting the PK similarity.

The mean denosumab serum concentration time-profiles within the PK-analysis set were overall comparable between ENZ215 and EU-Prolia groups.

DEN2 study (supportive):

The mean denosumab concentration-time profiles after the first IP dose (i.e., Day 1 until Month 6) were similar for the treatment groups, supporting PK similarity of test and reference product.

Several PK parameters were calculated for the first IP dose in study DEN2 (C_{max}, T_{max}, AUC_{0-1M}, AUC_{0-3M}, AUC_{0-6M},). Geometric mean values of C_{max}, AUC_{0-1M}, AUC_{0-3M} and AUC_{0-6M} were by about 2-13% higher in the ENZ215 group compared to the Prolia group. Nevertheless, the PK parameters were similar between the groups and support the PK similarity of the test and reference product in the osteoporosis patients.

PD

DEN1 study (supportive):

Overall, the secondary PD endpoint from study DEN1 (i.e., AUEC from baseline to Day 270 of CTX-1) was comparable between ENZ215 and EU-Prolia.

The overall shape of the mean %CfB-time profile of CTX-1 was similar between the ENZ215 and EU-Prolia groups.

DEN2 study (main PD study):

Biosimilarity in PD was also demonstrated in osteoporosis patients in study DEN2. "AUEC(0-6M) of serum sCTX" was defined as co-primary PD endpoint. The point estimate of the geometric LS means ratio (ENZ215/EU-Prolia) for this AUEC was 1.004 with the corresponding 95% CI being [0.9748, 1.0349]. Thus, the 95% CI was within the pre-specified equivalence range of [0.8, 1.25]. Although no justification for this arbitrary equivalence range was provided, the 95%CI is considered sufficiently close around unity to conclude on PD similarity.

PD similarity was further demonstrated by similar percent change from baseline in serum P1NP at each time-point from baseline until Month 6, including comparable serum P1NP concentration-time profiles of ENZ215 and EU-Prolia.

Efficacy

DEN2 study (main efficacy study):

The percentage change in BMD at Lumbar Spine (L1-L4 Region) measured by DXA from baseline to Month 12 was the co-primary efficacy endpoint in this study. The statistical analysis on the estimand based on the treatment policy strategy revealed that the LS-mean difference between the ENZ215 and the EU-Prolia group

was -0.183% with the corresponding 95% CI being [-0.9044, 0.5380]. Thus, the 95% CI was clearly within the pre-specified and accepted equivalence range of [-1.45%, 1.45%] and the co-primary efficacy endpoint was met. The analysis targeting the estimand based on the hypothetical strategy gave an estimated difference of -0.220 (95% CI: -0.932, 0.491) assuming that there were no BMD assessment delays and no use of prohibited medication.

Similarity in efficacy was further supported by the secondary efficacy endpoints. The percent change from baseline in bone mineral density at total hip at Months 6 and 12 was comparable between the groups. Similarly, the percent change from baseline in bone mineral density at the femoral neck at Month 6 was also comparable between the groups. Nevertheless, at Month 12 the increase in femoral neck BMD was statistically lower in the ENZ215 group compared to the Prolia group.

Safety

In the Phase 1 study with healthy male subjects, incidence of total TEAEs was slightly higher in the EU-Prolia group than in the ENZ215 and US-Prolia groups, with 39 (56.5%), 28 (41.2%), 32 (46.4%) of the subjects, respectively. However, this trend was not observed in the incidence of TEAEs related to the study drug by investigator judgement, which were reported in 5 (7.4%), 4 (5.8%), and 6 (8.7%) subjects, in the ENZ215, US-Prolia, and EU-Prolia groups, respectively. No healthy subject experienced a fatal event. One healthy subject in the EU-Prolia group experienced an SAE of a ligament rupture that resulted in hospitalization, considered as not related to study treatment. No TEAE leading to discontinuation of study treatment was reported in the healthy subject study.

As regards the postmenopausal patients with osteoporosis, the number of TEAEs in the double-blind phase of the study was higher in the ENZ215 group than in the Prolia group (441 events vs. 393 events) but affected a similar number and percentage of patients as those were reported in 164 (64.8%) patients of the ENZ215 group and in 157 (62.5%) patients of Prolia group. Additionally, no significant differences were observed when comparing incidence of severe TEAEs, and TEAEs assessed related to study treatment by the investigator (ENZ215: 25 events reported in 19 [7.5%], Prolia: 36 events reported in 23 [9.2%] patients, respectively). No significant differences were reported as regards the incidence of TESAEs (ENZ215: 23 events reported in 16 [6.3%], Prolia: 17 events reported in 15 [6.0%] patients, respectively). One death occurred in a patient in the Prolia group (that was not enrolled in the open label phase) on study day 385, caused by a TESEA of Covid-19, assessed that as not related to study treatment. Seven TEAEs graded severe were reported in the ENZ215 group in 5 patients (2.0%), whereas five were reported in the Prolia group in 5 patients (2.0%). TEAEs leading to treatment and study withdrawal were rare and with similar incidence between the two groups. TEAEs leading to treatment interruption were also rare and reported with similar incidence between the two groups. In the open-label phase, the number of TEAEs was higher in the ENZ215 group than in the Prolia group (36 events vs. 30 events) but affected a similar number and percentage of patients as those were reported in 23 (38.3%) patients of the ENZ215 group and in 23 (38.3%) patients of Prolia group; TEAEs considered related by the investigator were only reported in the ENZ215 group (3 events in 2 [3.3) patients). There were no SUSAR, SAE, fatal AE, or AE leading to study withdrawal, treatment withdrawal or treatment interruption reported during the open-label extension period.

Immunogenicity

Among the healthy study subjects, no significant differences in ADA incidence between the groups were reported. Incidence of NAbs was extremely low compared to total ADA incidence, e.g., on Day 28: 1 subject (1.4%) each in EU-Prolia and US-Prolia groups and no subject in the ENZ215 group. On Day 270, no subject had NAb positive across treatment groups. No significant differences in NAb incidence between the groups were reported.

Similarly to the healthy subjects, among the postmenopausal patients with osteoporosis, significant differences in ADA incidence between the groups were not observed for neither phase. Also, alike healthy subjects, incidence of NAbs was extremely low compared to total ADA incidence, e.g., only one subject in the Prolia group (0.4%) at Visit 5 (Day 30). Additionally, there were no significant differences in NAb incidence observable between the groups for neither phase and no patients had NAbs in either treatment group throughout the open-label extension period.

Only one case of drug hypersensitivity was reported in both studies, experienced by a patient in the ENZ215 group of the open-label phase.

3.3. Uncertainties and limitations about biosimilarity

Quality

No relevant concerns obviating a conclusion on biosimilarity remain at the quality level.

Clinical

In both the DEN1 and the DEN2 studies, a large number of protocol deviations occurred. In DEN1, 56% subjects had "minor" deviations and 13.5% of subjects had "major" deviations. During the double-blind period in DEN2, 68.8% reported "minor" deviations, while 8% reported one "major" deviation. 40% of patients had "minor" protocol deviations in the open-label period in DEN2. Upon request, the Applicant provided amended listings for multiple ambiguous protocol deviations, e.g., reasons leading to a classification into major and minor deviations, reasons leading to exclusion from particular analysis sets, which protocol deviations were considered as intercurrent events, why disallowed medication was not consistently handled, how many hours/days a procedure was "out of window".

PK

Although no sensitivity analysis has been performed for the primary PK endpoints (Cmax and AUC_{0-inf}; excluding all samples affected by protocol deviations related to PK laboratory assessments, study procedures, or visit schedules), the PK results from study DEN1 are considered sufficiently robust to support a conclusion of biosimilarity.

In some of the subjects' individual serum concentration profiles, a sudden fluctuation in concentration was observed around time point 120h in the DEN1 study. The Applicant provided a root cause analysis and sensitivity analyses, investigating samples/subjects with PK fluctuations. No single, clear root cause for the PK fluctuations could be identified. The Applicant's conclusion is considered acceptable: based on the sensitivity analyses and evaluations from clinical pharmacology, clinical operations, and bioanalysis, the observed fluctuations do not impact the biosimilarity conclusion between the test and reference products

PD

In the DEN2 study, an extreme percentage change from baseline value of -1505.9% was reported for one subject in the Prolia group at D30. Despite reviewing bioassay performance, sampling procedures, potential intercurrent events (none were reported for this patient), medical history, prior antiresorptive treatments, protocol deviations, and TEAEs, no definitive root cause could be identified by the Applicant. In the absence of documented osteoarthritis exacerbation or changes in medication with an influence on bone resorption (other than calcium), no clear explanation is apparent and the issue is not pursued further.

To address uncertainties regarding the impact of the high number of protocol deviations on the co-primary PD endpoint of study ALK22/ENZ215-DEN2, the Applicant submitted an additional sensitivity analysis. The outcome supports the robustness of the PD results and indicates that the protocol deviations observed in the DEN2 study did not compromise the conclusion of biosimilarity between ENZ215 and Prolia.

Efficacy

Results for the BMD secondary endpoints support the results of the co-primary endpoint, with the exception of the endpoint of BMD percentage change at the femoral neck at month 12, where results were in favour of Prolia over ENZ215. Also, for BMD change at the total hip at month 12, the trend was in favour of Prolia. This could be a chance finding considering the otherwise consistent efficacy results.

To address the impact of protocol deviations, the Applicant provided a sensitivity analysis using an adapted MMRM model targeting a hypothetical estimand for the primary endpoint. As with the PD analysis, the results support the robustness of the efficacy data and confirm that the protocol deviations did not affect the overall conclusion of biosimilarity between ENZ215 and Prolia.

Safety

Overall, the collected safety data appears indicative of comparable safety between the biosimilar candidate ENZ215 and the RMP Prolia.

Immunogenicity

The reported incidence of ADA-positive subjects was extremely high among the healthy study subjects and increased rapidly with 94.1% in ENZ215 and 100% in both EU-Prolia and US-Prolia groups as early as Day 16. Percentages declined after Day 147 until study end to some extent but remained relatively high regardless.

Similarly to the healthy subjects, the incidence of ADA-positive postmenopausal patients with osteoporosis in the double-blind phase was extremely high (e.g., at the second ADA sampling at Visit 5 (Day 30), a positive ADA confirmatory analysis in 235 (93.6%) in the ENZ215 group and 228 (92.3%) in the Prolia group). In the open-label extension phase, at Visit 10 (Day 450) all patients (i.e., in both groups) had confirmed ADA. The incidence was reported to be lower to some extent at Visit 7 (Day 180) and Visit 9 (Day 360) in the double-blind phase, which can be expected as those were the visits with utmost timely distance to the last treatment administration. This was observed also in the results of the open-label extension phase at Visit 11 (Day 540): 55 (91.7%) patients in the ENZ215 group and 50 (83.3%) patients in the Prolia group had confirmed ADA.

This significantly higher incidence of total ADA in all groups and studies compared to ADA incidence reported in the studies for MAA of Prolia could be considered concerning, however, could be explained by being a result of the high sensitivity of the applied assay (6.44 ng/mL cut-off) or, potentially, being attributed to target interference of sRANKL. Upon request, the Applicant provided data and relevant discussion, from which it can be concluded that the PK parameters are largely comparable between ENZ215 and US Prolia across both ADA-negative and ADA-positive subgroups.

3.4. Discussion on biosimilarity

Quality

Provided resolution of the concerns raised on the analytical biosimilarity exercise, on a quality level, ENZ5121 can be regarded biosimilar to Prolia.

Clinical

Study conduct

In both the DEN1 and the DEN2 studies, a large number of protocol deviations occurred. In DEN1, 56% subjects had "minor" deviations and 13.5% of subjects had "major" deviations. During the double-blind period in DEN2, 68.8% reported "minor" deviations, while 8% reported one "major" deviation. 40% of patients had "minor" protocol deviations in the open-label period in DEN2. The large number of protocol deviations is of major concern. The Applicant provided a more exhaustive summary table and protocol deviation listings which are considered sufficient, and no concern remains.

PK

In *study DEN1,* conducted in healthy male volunteers, PK similarity was formally demonstrated between ENZ215 and EU-Prolia as the 90% CIs for the GLSM of the ratio test/reference for the primary PK parameters ($AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and C_{max}) were fully contained within the predefined bioequivalence limits of [80.00% to 125.00%]. Furthermore, no notable treatment differences were observed in the secondary PK parameters ($pAUC_{0\text{-}D28}$, T_{max}, $T_{1/2}$, and CL/F).

Although no sensitivity analysis for the primary PK endpoints (Cmax and AUC_0–inf; excluding all samples affected by protocol deviations related to PK laboratory assessments, study procedures, or visit schedules), was provided, the PK results from study DEN1 are considered sufficiently robust to support a conclusion of biosimilarity.

In some of the subjects' individual serum concentration profiles, a sudden fluctuation in concentration was observed around time point 120h. Sensitivity analyses showed that the 90% CIs for all parameters remained fully within the predefined bioequivalence acceptance range of 80.00% to 125.00%. The Applicant also provided a root cause analysis which identified two bioanalysis-related problems involving two subjects. For the remaining fluctuations, no single root cause could be determined. Additionally, a literature review was provided. Based on the sensitivity analyses and evaluations from clinical pharmacology, clinical operations, and bioanalysis, the observed fluctuations are considered not to have a meaningful impact on the bioequivalence conclusion between ENZ215 and EU-Prolia.

PK data from *study DEN2* conducted in female osteoporosis patients further support PK similarity of the test and reference product. The mean denosumab concentration-time profiles for the whole study period were similar for the treatment groups. Additionally, although there was an approximately 2-13% higher exposure in the PK parameters calculated for the first IP dose (C_{max}, $AUC_{0\text{-}1M}$, $AUC_{0\text{-}3M}$ and $AUC_{0\text{-}6M}$) in study DEN2, the exposure was overall similar between the treatment groups, supporting the PK similarity of the test and reference product in the osteoporosis patients.

PD

PD similarity is supported by both clinical studies, DEN1 and DEN2, and is specifically demonstrated in study *DEN2*.

In *study DEN1*, the secondary PD endpoint AUEC(0-D270) for CTX-1 was similar between the two treatment groups.

In study DEN2, biosimilarity in PD was also demonstrated in osteoporosis patients in study DEN2. The biosimilarity criterion for the co-primary PD endpoint "AUEC(0-6M) of serum sCTX" was met. The point

estimate of the geometric LS means ratio (ENZ215/EU-Prolia) for AUEC was 1.004 with the corresponding 95% CI being [0.9748, 1.0349]. Thus, the 95% CI was within the pre-specified equivalence range of [0.80, 1.25]. Although no justification for this arbitrary equivalence range was provided, the 95%CI is considered sufficiently close around unity to conclude on PD similarity. The primary PD analysis was performed on the PD set, which excluded patients with major protocol deviations affecting sCTX or sP1NP measures. The Applicant provided revised versions of the analysis using the ITT set as analysis set by applying multiple imputation techniques which support the analysis using the PD analysis set.

PD similarity was further demonstrated by similar percent change from baseline in serum P1NP at each time-point from baseline until Month 6 in the DEN2 study, including comparable serum P1NP concentration-time profiles of ENZ215 and EU-Prolia.

Efficacy

Similarity regarding efficacy was shown in *study DEN2*. The co-primary efficacy analysis on the percent change from baseline in LS-BMD at the Lumbar Spine (L1-L4 Region) at Month 12 was met, as the difference between the ENZ215 and the EU-Prolia group with the corresponding 95% CI was within the pre-specified and accepted acceptance range [-1.45%, 1.45%]. The statistical analysis of the treatment policy estimand revealed that the difference between the ENZ215 and the EU-Prolia group was -0.183% with the corresponding 95% CI being [-0.9044%, 0.5380%]. To address protocol deviations, the Applicant provided a sensitivity analysis for the primary PD endpoint, which supported results of the co-primary efficacy analysis.

Efficacy analyses of the BMD at total hip at Month further support the similarity in efficacy between the test and reference product. Nevertheless, at Month 12 the increase in femoral neck BMD was statistically lower in the ENZ215 group compared to the Prolia group, which in light of the other EPs being clearly comparable, are likely a chance finding.

Safety

Overall, the collected safety data appear indicative of comparable safety between the biosimilar candidate ENZ215 and the RMP Prolia.

Immunogenicity

While the reported incidence of ADA-positive subjects was extremely high among both the healthy study subjects and the postmenopausal patients with osteoporosis, no significant differences in ADA incidence between the respective groups in those studies were observed. As regards NAbs, which are seen more critical due to the neutralization of study drug, the incidence was extremely low compared to total ADA incidence for both healthy subjects and patients, which mitigates potential concerns. The Applicant presented data on ADA titres for both studies and also denosumab serum concentrations and changes in lumbar spine BMD by ADA status for both treatment groups in PMO patients. Those analyses did not indicate a negative effect of ADA titer level on efficacy or safety. Moreover, only one case of drug hypersensitivity was reported in both studies, experienced by a patient in the ENZ215 group of the open-label phase.

3.5. Extrapolation of safety and efficacy

ENZ215 was developed as a biosimilar product to Prolia. The mechanism of action is identical to the reference product. The mechanism of action is also identical across all indications. The monoclonal antibody denosumab targets and binds to RANKL, thus preventing interaction of RANKL with RANK. Block of interaction of RANKL

with RANK leads to reduced osteoclast formation and function. Thus, bone resorption and cancer induced bone destruction is decreased.

The desired pharmacological action of denosumab occurs invariably in the bony tissue, through prevention of generalized bone resorption in primary or secondary osteoporosis, or local bone resorption and destruction around bone metastases. Thus, based on the same mechanism of action, extrapolation to all indications might be allowed.

The extrapolation is further supported by the fact that the known PK, safety and immunogenicity profile of denosumab (as summarized in the product information for Prolia) is comparable across the approved indications and patient populations.

Furthermore, the clinical data were derived from healthy male volunteers and female osteoporosis patients. These are regarded sensitive populations in terms of evaluating biosimilarity of ENZ215 and the reference product.

Based on the above, the safety and efficacy profile of ENZ215 as assessed in the PMO indication can be extrapolated to all indications applied for Osqay.

3.6. Conclusions on biosimilarity and benefit risk balance

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

4. Recommendations

Outcome

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

> Treatment of osteoporosis in postmenopausal women and in men at increased risk of fractures. In postmenopausal women denosumab significantly reduces the risk of vertebral, non-vertebral and hip fractures.
>
> Treatment of bone loss associated with hormone ablation in men with prostate cancer at increased risk of fractures (see section 5.1). In men with prostate cancer receiving hormone ablation, denosumab significantly reduces the risk of vertebral fractures.
>
> Treatment of bone loss associated with long-term systemic glucocorticoid therapy in adult patients at increased risk of fracture (see section 5.1).

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

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

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

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

- **Risk Management Plan (RMP)**

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

An updated RMP should be submitted:

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

- **Additional risk minimisation measures**

The MAH shall ensure that a patient reminder card regarding osteonecrosis of the jaw is implemented.