CLINICAL TRIAL PROTOCOL

CLINICAL TRIAL PHASE- 1

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

Protocol Number – ALK22/ENZ215-DEN1

EUDRACT No: 2021-004177-32

Version 4.0, Dated 03/Oct/2022

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CONFIDENTIAL

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1. STUDY INFORMATION

1.1. STUDY CONTACT INFORMATION DETAILS

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1.2. SPONSOR'S DECLARATION

Study Title: 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

Protocol Number: ALK22/ENZ215-DEN1

Version 4.0, 03/Oct/2022

I, the undersigned, have read and understood this protocol and hereby agree to conduct the study in accordance with the approved protocol complying with all the requirements regarding the obligations of Sponsor and all other pertinent requirements of the Declaration of Helsinki (Ethical principles for medical research involving human subjects, revised by the 64th WMA General Assembly, Brazil, October 2013), which are consistent with the ICH-GCP E6(R2) guidelines and applicable regulatory requirements. I agree to provide compensation in the case of clinical trial related injury or death for which subjects are entitled to as per applicable regulatory guidelines. I also agree to comply with cGMP requirements for the study drug for subject consumption.

ALKEM LABORATORIES LIMITED ★ MUMBAI, INDIA ★

03/10/2022.

Signature: **Date:**

Dr. Akhilesh Sharma
President & Chief Medical Officer,
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1.3. INVESTIGATOR'S AGREEMENT

Study Title: 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

Protocol Number: ALK22/ENZ215-DEN1

Version 4.0, 03/Oct/2022

I, the undersigned, have read and understood this protocol and hereby agree to conduct the study in accordance with the approved protocol complying all the requirements regarding the obligations of Investigators and all other pertinent requirements of the Declaration of Helsinki (Ethical principles for medical research involving human subjects, revised by the 64th WMA General Assembly, Brazil, October 2013), which are consistent with the ICH-GCP E6 (R2) guidelines and applicable regulatory requirements.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than evaluation or conduct of the clinical investigation without the prior consent of Sponsor. I understand that the Sponsor may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately in writing to the Sponsor.

I further agree to ensure that all associates assisting in the conduct of this study are well informed regarding their obligations and confirm to conduct this study under my direction.

Signature: **Date:**

Investigator's name:

Site address:

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3. LIST OF ABBREVIATIONS

ADAs	:	Antidrug antibodies
AE	:	Adverse Event
ALP	:	Alkaline Phosphatase
ALT	:	Alanine aminotransferase
ANCOVA	:	Analysis of covariance
AST	:	Aspartate aminotransferase
$AUC_{0\text{-inf}}$	:	Area under the drug concentration-time curve from time 0 to infinity
$AUC_{0\text{-t}}$		Area under the drug concentration-time curve from day 0 to day 270
$AUC_{0\text{ - Day 28}}$	:	Partial area under the drug concentration-time curve from time 0 (pre-dose) to day 28
AUEC	:	Area under the effect curve
$AUEC_{0\text{-t}}$	:	The area under the serum effect versus time curve, from time 0 to the last measurable time point, as calculated by the log-linear trapezoidal method
BMI	:	Body mass index
BMD	:	Bone mineral density
BUN	:	Blood urea nitrogen
CDMS	:	Clinical Data Management System
CFR	:	Code of Federal Regulation
C_{max}		Maximum observed drug concentration
cGMP	:	Current Good Manufacturing Practice
CI	:	Confidence Interval
CL/F	:	Apparent systemic clearance
COA	:	Certificates of analysis
COVID-19	:	Coronavirus disease 2019
CTCAE	:	Common terminology criteria for adverse events
CTX-1	:	C-terminal telopeptide type-1
CV	:	Coefficient of variation
EC	:	Ethics Committee
ECG	:	Electrocardiogram
eCTD	:	electronic Common Technical Document
eCRF	:	Electronic Case Record Form
EDC	:	Electronic Data Capture
EOS	:	End of Study
EU	:	European Union
FDA	:	Food and Drug Administration of the United States
GCP	:	Good Clinical Practice
GDPR	:	General Data Protection Regulation
GVP	:	Good Pharmacovigilance Practices
Hb	:	Hemoglobin
HBV/ HBsAg	:	Hepatitis B Virus/ Hepatitis B surface Antigen

HCV	:	Hepatitis C Virus
HIV	:	Human Immunodeficiency Virus
ICF	:	Informed Consent Form
ICH	:	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IEC	:	Independent Ethics Committee
IP	:	Investigational Product
IRB	:	Institutional Review Board
MHRA	:	Medicines and Healthcare products Regulatory Agency
mL	:	Milliliter
MVF	:	Multiple Vertebral Fractures
NAB	:	Neutralizing antibodies
ONJ	:	Osteonecrosis of the jaw
PFS	:	Pre-filled syringe
PD	:	Pharmacodynamic
PK	:	Pharmacokinetic
RANKL	:	Receptor activator of nuclear factor kappa-B ligand
RBC	:	Red Blood Cell
SAE	:	Serious Adverse Event
SARS-CoV-2	:	Severe acute respiratory syndrome coronavirus-2
SAP	:	Statistical Analysis Plan
SC	:	Subcutaneous
SmPC	:	Summary of Product Characteristics
SOP	:	Standard Operating Procedure
T_{max}		Time to reach C_{max}
$T_{1/2}$		Terminal elimination half-life
ULN	:	Upper Limit of Normal
USFDA	:	United States Food and Drug Administration
WBC	:	White Blood Cell
WMA	:	World Medical Association

4. GLOSSARY

Assessment	:	A procedure used to generate data required by the study
Biosimilar	:	Similar biologic means a biological product which is similar in terms of quality, safety and efficacy to reference biological product licensed or approved by regulatory agency, or any innovator product approved in International Council of Harmonization (ICH) member countries
Concomitant medication	:	A concomitant medication (con-med) is a drug or biological product, other than the investigational product, taken by the subject during clinical trial participation
Eligible subjects	:	Subjects who fulfill all the inclusion criteria and none of the exclusion criteria are considered as eligible subjects for the study
End of study (EOS) assessment	:	EOS will be performed on day 270 (week 39) or at the time of early discontinuation/withdrawal of the subject
Enrolled subjects	:	The subjects who sign the informed consent form (ICF) and fulfill the study selection criteria will be considered as enrolled subjects in the study
Protocol deviation/ violation	:	Any change, divergence or departure from the study design or procedure defined in the protocol
Randomization	:	A method based on chance alone by which the subjects are assigned to a treatment group
Screening Period	:	The process to determine that the subject is eligible to participate in the study
Study duration	:	The duration from screening period till EOS assessment visit
Study period	:	The period (day 1 to day 270) during which all the study related assessments are carried out including investigational product administration

5. PROTOCOL SYNOPSIS

Title	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
Protocol number	ALK22/ENZ215-DEN1
Trial location	UK Belfast, Poland, Warszawa, Bulgaria Sofia and or India Bengaluru
Development phase	Phase 1
Study objective(s)	**Primary objective:** • To demonstrate bioequivalence between ENZ215 and EU- and US-sourced Prolia® using PK parameters **Secondary objective(s):** • To compare the serum PK profile of ENZ215 and EU- and US-sourced Prolia® • To compare the serum CTX-1 profile of ENZ215 and EU- and US-sourced Prolia® • To compare the immunogenicity profile of ENZ215 and EU- and US-sourced Prolia® • To compare the safety and tolerability profile of ENZ215 and EU- and US-sourced Prolia®
Study endpoint(s)	**Primary endpoint:** • Maximum observed drug concentration (C_{max}), area under the drug concentration-time curve from day 0 to day 270 (AUC_{0-t}) and area under the drug concentration-time curve from time 0 to infinity (AUC_{0-inf}) of ENZ215 and EU- and US-sourced Prolia® **Secondary endpoint(s) (Pharmacokinetics):** • Partial area under the drug concentration-time curve from time 0 (pre-dose) to day 28 • Time to reach C_{max} (t_{max}) • Terminal elimination half-life ($t_{1/2}$) • Apparent systemic clearance (CL/F)

	Secondary endpoint(s) (Pharmacodynamics): • Area under the effect curve (AUEC) from time 0 to Day 270 for serum CTX-1 percent inhibition **Safety endpoint(s):** • 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)
Study design	A randomized (1:1:1), double-blind, three-arm, parallel-group, single-dose study in healthy adult male volunteers
Sample size	It is assumed that the true ratio for AUC_{0-t}, AUC_{0-inf} and C_{max} between ENZ215, EU-sourced Prolia® and US-sourced Prolia® is 0.95 and the between subject coefficient of variation (CV) for AUC_{0-t}, AUC_{0-inf} and C_{max} 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 AUC_{0-t}, AUC_{0-inf} and C_{max} 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.
Test Product, Dose, Route of Administration and Regimen	ENZ215 (Biosimilar Denosumab) manufactured by Enzene Biosciences Ltd. A single-dose, 60 mg SC administration
US-Reference Product, Dose, Route of Administration and Regimen	US-sourced Prolia® (denosumab) injection A single-dose, 60 mg SC injection administration
EU-Reference Product, Dose, Route of Administration and Regimen	EU-sourced Prolia® injection (denosumab) A single-dose, 60 mg SC injection administration

Study duration	The study duration will be approximately 16 months (i.e. 6 months of recruitment period, 4 weeks of screening period and approximately 39 weeks (270 days) of study period). • **Screening Period:** It will last up to 4 weeks during which the subject will be assessed for eligibility in the study • **Study Period:** It will last for 270 days (about 39 weeks) where subjects will receive either ENZ215 or EU- or US-sourced Prolia® (60 mg - single dose subcutaneous injection), and all study related assessments will be carried out • **EOS Assessment** will be performed on day 270 (week 39)
Key inclusion criteria	The subjects will be included in the study based on the following 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/m^2 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: A 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).
Key exclusion criteria	The subjects will be excluded from the study based on the following 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, 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 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).
Study specific Discontinuation/ Withdrawal criteria	The subject may be withdrawn/discontinued from the study due to the following reasons: 1. The subject suffers from significant inter-current illness or undergoes surgery during the course of the study 2. Subject's voluntary withdrawal of consent 3. Subject non-compliance to pre- and post-dose requirements 4. Protocol violation 5. In Investigator's opinion it is not in the subject's best interest to continue 6. The subject is found to conceal important medical history which in opinion of Investigator may compromise his safety during participation in this study 7. Subjects experiencing signs or symptoms of systemic hypersensitivity reactions, anaphylactic or other clinically significant allergic reactions during the study 8. Any AE or SAE which requires discontinuation of subject in the opinion of Investigator 9. Study termination by the Sponsor 10. Any other justifiable reason, which should be adequately documented
Study Procedure/ Methodology	This is a randomized, double-blind, three-arm, parallel-group, single-dose study to demonstrate bioequivalence of ENZ215 and EU- and US-sourced Prolia after a single 60-mg dose administered subcutaneously in healthy adult male volunteers. Subjects will be screened for enrolment in the study from day -28 to day -1 before randomization. Approximately 207 subjects will be enrolled into 3 groups (69 in each group) in parallel. The subjects may be enrolled in multiple groups at the site. All the eligible subjects will be randomized (1:1:1) to receive a single SC 60-mg dose of either ENZ215 or EU- or US-sourced Prolia on Day 0/1 Eligible subjects will be admitted/check-in to the phase 1 study site at least 10 hours prior to dosing on day 1 and will be discharged/check out from phase 1 study site on day 2 after PK sample collection (in-subject period). All screening and on-study blood samples will be processed and sent to local or central laboratory as applicable as defined in the lab manual. A total of twenty four (24) blood PK samples of 2.5 mL each will be collected from each subject in the study. Blood samples for PK analysis will be collected at 0 hour (pre-dose), and at 1, 4, 8, and 12 hours (day 1), day 2 (24 hours), day 3 (48 hours), day 4 (72 hours), day 5 (96 hours), day 6 (120 hours), day 8 (168 hours), day 10 (216 hours), day 12 (264 hours), day 16 (360 hours), day 21 (480 hours), day 28 (648

	hours) (week 4), day 42 (984 hours) (week 6), day 63 (1488 hours) (week 9), day 90 (2136 hours) (week 13), day 119 (2832 hours) (week 17), day 147 (3504 hours) (week 21), day 180 (4296 hours) (week 26), day 224 (5352 hours) (week 32) and at the end of study (day 270 (6456 hours) (week 39) post-dose. The details pertaining to the sample collection window period is provided in study visit schedule table section 5.2. Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose samples up to day 2 will be collected within ± 10 minutes, within ± 2 hour from day 3 to day 21, within ± 1 day on day 28, within ± 3 days from day 42 to day 180 and within ±5 days from day 224 to day 270. A total of sixteen (16) blood for serum CTX-1 estimation of 3.5 mL each will be collected from each subject in the study. Blood samples for serum CTX-1 assessment will be collected at 0 hour (pre-dose), and at day 2 (24 hours), day 3 (48 hours), day 4 (72 hours), day 5 (96 hours), day 6 (120 hours), day 8 (168 hours), day 10 (216 hours), day 12 (264 hours), day 16 (360 hours), day 21 (480 hours), day 28 (648 hours) (week 4), day 63 (1488 hours) (week 9), day 119 (2832 hours) (week 17), day 180 (4296 hours) (week 26) and at the end of study (day 270 (6456 hours) (week 39) post-dose). 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. The details pertaining to the sample collection window period is provided in study visit schedule table section 5.2 Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose sample of day 2 will be collected within ± 10 minutes and samples from day 3 to day 21 will be collected within ± 2 hours, within ± 1 day on day 28, from day 42 to day 180 within ± 3 days and from day 224 to day 270 within ±5 days. A total of ten (10) blood Immunogenicity assessment samples of 5.0 mL for NAB (Neutralizing antibodies) and ADA (Anti-Denosumab antibody) will be collected from each subject in the study. Blood samples for Immunogenicity assessment will be collected at 0 hour (pre-dose), day 8 (168 hours), day 16 (360 hours), day 28 (648 hours) (week 4), day 63 (1488 hours) (week 9), day 90 (2136 hours) (week 13), day 119 (2832 hours) (week 17), day 147 (3504 hours) (week 21), day 180 (4296 hours) (week 26) and at the end of study (day 270 (6456 hours) (week 39) post-dose). The details pertaining to the sample collection window period is provided in study visit schedule table section 5.2. Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose sample of day 2 will be collected within ± 10 minutes and samples from day 3 to day 21 will be collected within ± 2 hours, within ± 1 day on day 28, from day 42 to day 180 within ± 3 days and from day 224 to day 270 within ±5 days.

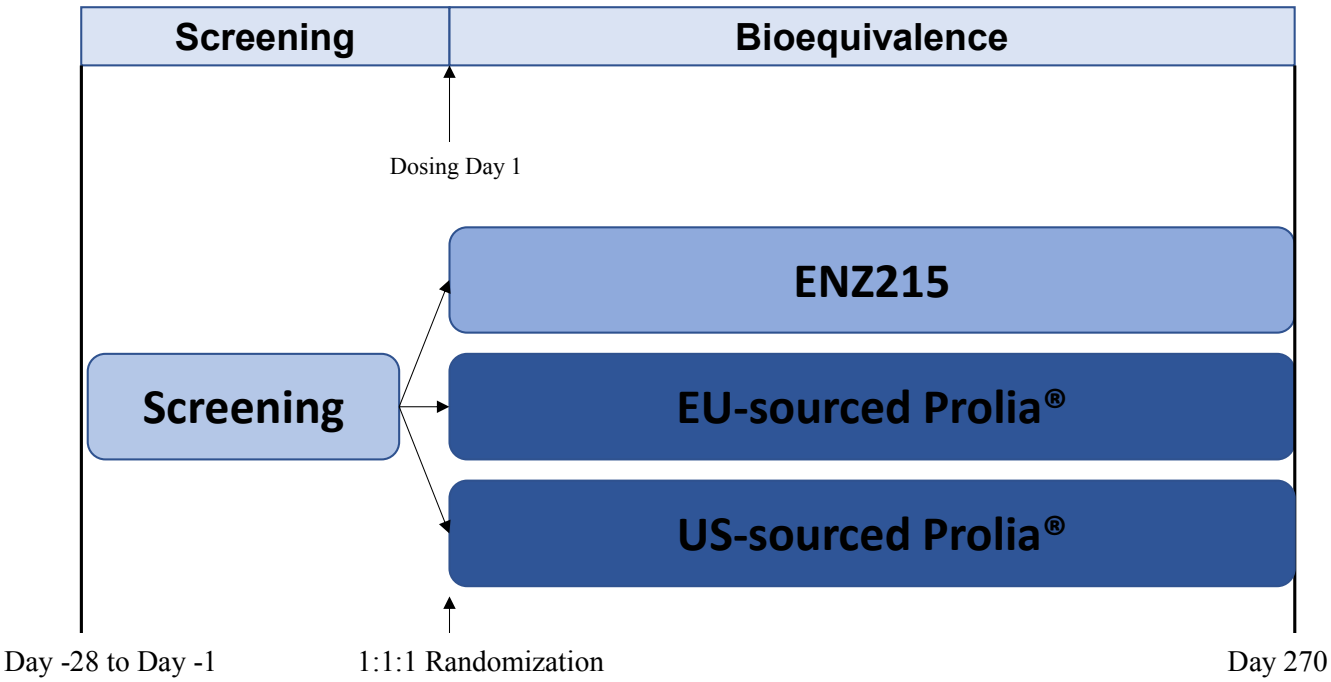
	For subjects with confirmed positive test for COVID 19, blood samples on and after day 3 (48 hours) may be collected from subjects at their place by third party appointed by CRO/ Sponsor. End of study safety assessment will be performed on day 270 (week 39). Safety assessment will be done throughout the study.
Investigational Product Administration procedure	Based on the randomization schedule, investigational product (either ENZ215 or EU- or US-sourced Prolia) will be administered to the subjects at a dose of 60 mg (single-dose) subcutaneously on day 1 into the upper thigh. **Special precautions for disposal and handling** • Before administration, the solution should be inspected. Do not inject the solution if it contains particles, or is cloudy or discoloured. • Do not shake. • To avoid discomfort at the site of injection, allow the pre-filled syringe (PFS) to reach room temperature (up to 25°C) before injecting and inject slowly. This generally takes 15 to 30 minutes. Do not warm investigational product in any other way. • Inject the entire content of the pre-filled syringe.
Collection, processing and shipment of biological samples	A separate plan will be prepared for collection, processing and shipment of biological samples. The information on sample handling will be included in Study Specification sheet/ lab manual/equivalent document as per SOPs [i.e. sample collection, processing and storage requirements (i.e. sample type, collection time, collection tubes, centrifuge duration, speed and temperature requirement, aliquoting, storage temperature, shipments condition etc].
Bioanalysis	Anti-Denosumab antibody and Neutralizing antibody assessment will be done at bioanalytical lab. Serum concentrations of denosumab and CTX-1 will be measured at bioanalytical lab by a validated analytical method. The procedure for sample handling, storage and analysis for the above samples will be provided in the Study Specification Sheet/ lab manual/equivalent document.
Safety Assessment	Safety assessment will be carried out as outlined in Section 11 of this protocol and adverse event (AE) or Serious Adverse Event (SAE) information will be collected and reported as mentioned in Section 12 of the protocol. Adverse events and Serious adverse events as and when occur, will be properly recorded, evaluated, managed and reported from signing informed consent till EOS assessment visit. Safety will be evaluated throughout the study based on complete physical examination, including signs and symptoms of COVID-19, adverse event monitoring, vital signs (blood pressure, pulse rate, respiratory rate and body temperature), 12-lead ECG, Anti-Denosumab antibody and Neutralizing antibody assessment and laboratory investigations. Any clinically significant abnormalities including those

	present prior to the start of study medication will be recorded as medical history. Adverse events persisting at the end of the study will be followed up by the Investigator until resolution or until a clinically stable endpoint is reached. All AE's will be summarized using appropriate medical coding dictionary.
Pharmacokinetics Assessments:	Pharmacokinetics: The following primary PK parameters will be calculated for Denosumab in serum, as appropriate: C_{max}, AUC_{0-t} and AUC_{0-inf}. The following secondary PK parameters will be calculated for Denosumab in serum, as appropriate: $AUC_{0-28\ Day}$, T_{max}, T½, and CL/F.
Statistical analysis	All data will be listed and descriptive analysis along with appropriate graphs will be provided by treatment group. Categorical data will be summarized using absolute and relative frequencies (counts and percentages) and the summary measures for continuous data will include the number of observations, the arithmetic mean, standard deviation, minimum, median, maximum, CI, geometric mean, and CV as appropriate. **Primary Endpoint Analysis:** Analyses of variance (ANOVA) will be performed on the natural log (ln)-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} PK parameters, with treatment as a fixed effect. 90% confidence intervals (CIs) for the geometric mean ratios (GMRs), ENZ215 (test)/EU sourced Prolia (Reference A) and ENZ215 (test)/US Sourced Prolia (Reference B) will 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 will be met if the 90% CIs for the GMRs of C_{max}, AUC_{0-t} and AUC_{0-inf} of denosumab for the Test (Treatment A) to the Reference (Treatment B i.e US Source Prolia & Treatment C i.e. EU Source Prolia) fall within the limit of 80.00 to 125.00%. **Secondary Endpoints Analyses:** ***Pharmacokinetics:*** $pAUC_{0-28days}$, $t_{1/2}$ and CL/F will be compared between ENZ215 and Prolia using tests after log-transformation wherever appropriate. The non-parametric analysis will be used for the comparison of t_{max} between ENZ215 and Prolia. ***Pharmacodynamics:*** The AUEC will be calculated as the area under the effect curve from baseline until CTX-1 values return to baseline for the first time. An ANCOVA will 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 will be based upon the 95% confidence intervals for the ratio of the geometric means (ENZ215 and Prolia) for AUEC of baseline-corrected serum CTX-1 (i.e. % change from baseline), which have to be contained entirely within the pre-specified limits of 0.80-1.25. ***Safety:*** Safety parameters such as incidence of adverse events, clinically significant changes in physical examination findings, safety laboratory analytes (serum chemistry, hematology, and urinalysis), vital signs, 12-lead electrocardiogram, Anti-Denosumab antibody and Neutralizing antibody assessment will be descriptively summarized, for the treatment groups as appropriate. Analysis and descriptive methodologies will be further elaborated in the main body of the protocol as well as in the Statistical Analysis Plan for this study.
Ethical Considerations	The study will commence only after obtaining written approval from applicable regulatory authority and ethics committee. The clinical trial will be conducted as per applicable regulatory requirements, ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) E6(R2) 'Guideline for Good Clinical Practice' and the Declaration of Helsinki (Brazil) 2013. The study will be registered on applicable clinical trial registry.

5.1. STUDY FLOW CHART

Figure 5.1: Study Flow Chart



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

5.2. STUDY VISIT SCHEDULE

Assessments	Screening Period	Study Period																				EOS Assessment
Day	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**	**16**	**21**	**28**	**42**	**63**	**90**	**119**	**147**	**180**	**224**	**270**
Week	**-**	**-**			**-**									**4**	**6**	**9**	**13**	**17**	**21**	**26**	**32**	**39**
Visit window	**N/A**	**NA**			**± 2 hour**									**±1 day**	**±3 days**						**±5 days**	
Visits	**1**	**2**			**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Informed consent	X																					
Inclusion/exclusion criteria	X	X																				
Demographics	X																					
BMI	X																					
Medical/surgical history	X																					
Prior medication history	X																					
Physical examination	X	X		X[a]																		X
COVID-19 signs and symptoms	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oral examination[q]	X	X												X			X			X		X
Vital signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alcohol test (breath analyzer or any suitable test)	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessments	Screening Period	Study Period																				EOS Assessment
Day	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**	**16**	**21**	**28**	**42**	**63**	**90**	**119**	**147**	**180**	**224**	**270**
Week	**-**	**-**			**-**									**4**	**6**	**9**	**13**	**17**	**21**	**26**	**32**	**39**
Visit window	**N/A**	**NA**			**± 2 hour**									**±1 day**	**±3 days**						**±5 days**	
Visits	**1**	**2**			**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Urine screen for drugs of abuse[c]	X	X																				
Urine screen for cotinine test	X	X									X			X	X	X	X	X	X	X	X	X
COVID-19 test[m]	X	X																				
Admission to phase-1 study site		X																				
Randomization		X[e]	X[e]																			
Adverse event reporting[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study drug administration[e]			X																			
Discharge/Check out from phase-1 study site				X																		
12-lead ECG	X																					X
Hematology[f, n]	X																					X
HIV, HBsAg, HCV	X																					

Assessments	Screening Period	Study Period																				EOS Assessment
Day	**-28 to -1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**	**16**	**21**	**28**	**42**	**63**	**90**	**119**	**147**	**180**	**224**	**270**
Week	**-**	**-**			**-**									**4**	**6**	**9**	**13**	**17**	**21**	**26**	**32**	**39**
Visit window	**N/A**	**NA**			**± 2 hour**									**±1 day**	**±3 days**						**±5 days**	
Visits	**1**	**2**			**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Serum chemistry[g,n]	X																					X
Serum chemistry – albumin-adjusted calcium only[h, n]			X	X	X			X	X	X	X									X		X
Urinalysis[i]	X																					X
Pharmacokinetics[j, n, p]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacodynamics[k. n, p] : serum CTX-1 serial collection			X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X
Anti-drug antibodies and Neutralizing antibody[l, n, p]			X						X			X		X		X	X	X	X	X		X
Injection site assessment[o]			X																			

D = day, W = week, BP = blood pressure, EOS = end of study, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus, HIV = human immunodeficiency virus, PK = pharmacokinetic, PR = pulse rate, RR = respiratory rate.

a. At the time of discharge/check out

b. Vital signs will be recorded in supine position after 5 minutes of rest: PR (beats/minute), BP measurement (mm of Hg), RR (breaths/minute), and body temperature (°C).

c. Includes Benzodiazepines, Cannabinoids, Amphetamine, Cocaine, Barbiturates, Morphine
d. Recording of adverse events will start after signing of informed consent up to EOS (day 270).
e. Randomization can be done either on day 0/ day1, however, IP administration must be done on day 1. Independent study personnel shall monitor the dosing via subcutaneous administration route.
f. Hematology includes total leukocyte count (WBC count), total erythrocyte count (RBC count), hemoglobin, platelet count, and differential (absolute and percentage) WBC count
g. Serum chemistry includes liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], total bilirubin, albumin and total proteins), kidney function tests (blood urea nitrogen [BUN@], creatinine), electrolytes (sodium, potassium, chloride), glucose, serum calcium, albumin-adjusted calcium, magnesium, and phosphorus.
h. Albumin-adjusted calcium will be analyzed on Days 1 (pre-dose), 2, 3, 6, 8, 10, 12, 180, and 270
i. Urine analysis includes: Color, transparency, pH, specific gravity, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, leucocyte esterase, and protein; Microscopic examination: RBC, leucocytes, epithelial cells and bacteria, crystals.
j. PK time points: 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)
k. Serum CTX-1 time points: 0 hour (pre-dose), and at 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 63 (week 9), day 119 (week 17), day 180 (week 26), and at end of study (day 270 [week 39] post-dose). 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.
l. Immunogenicity time points: 0 hour (pre-dose), day 8, day 16, day 28 (week 4), day 63 (week 9), day 90 (week 13), day 119 (week 17), day 147 (week 21), day 180 (week 26) and at end of study (day 270 (week 39) post-dose)
m. COVID testing will be performed in line with the clinical site's current COVID prevention of infection management strategy and applicable local government/health guidelines
n. Subjects with confirmed positive test for COVID 19, blood samples on and after day 3 (48 hours) may be collected from subjects at their place by third party appointed by CRO/ Sponsor.
o. Injection site will be assessed for local reaction post-administration
p. In case of missed sample, compensatory samples will be collected as applicable in consultation with concerned scientist (PK, PD & AD), PI and Medical Monitor
q. In case of any dental findings (which are clinically significant as per investigator's judgement) recommendation for appropriate preventive dentistry shall be made if applicable

@Note: BUN will be reported in the form of Urea in mmol/L.

6. BACKGROUND AND INTRODUCTION

6.1. Overview of Biosimilar Denosumab:

Biosimilar Denosumab (ENZ215) has been developed as a similar biological medicinal product to Prolia® (denosumab, Amgen Inc.).

Biosimilar Denosumab and Prolia® have identical primary, secondary and tertiary structure and the active substance for both products is denosumab; a novel antiresorptive agent that inhibits osteoclast-mediated bone resorption through a different pathway.

Denosumab is a human IgG2 monoclonal antibody with affinity and specificity for human RANKL (receptor activator of nuclear factor kappa-B ligand); produced using genetically engineered mammalian (Chinese hamster ovary (CHO)) cells.

In regards to the mechanism of action, Biosimilar Denosumab, binds to RANKL and prevents activation of RANK receptor on the surface of osteoclasts and their precursors. Thus, inhibiting osteoclast formation, function, and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone.

6.2. Potential Risk and Benefits of Innovator Denosumab

WARNINGS AND PRECAUTIONS

Drug Products with Same Active Ingredient

Hypersensitivity

Clinically significant hypersensitivity including anaphylaxis has been reported with Prolia. Symptoms have included hypotension, dyspnea, throat tightness, facial and upper airway edema, pruritus, and urticaria.

Hypocalcemia and Mineral Metabolism

Hypocalcemia may be exacerbated by the use of Prolia. Pre-existing hypocalcemia must be corrected prior to initiating therapy with Prolia. In patients predisposed to hypocalcemia and disturbances of mineral metabolism (e.g. history of hypoparathyroidism, thyroid surgery, parathyroid surgery, malabsorption syndromes, excision of small intestine, severe renal impairment [Creatinine clearance < 30 mL/min] or receiving dialysis, treatment with other calcium-lowering drugs), clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended within 14 days of Prolia injection. In some postmarketing cases, hypocalcemia persisted for weeks or months and required frequent monitoring and intravenous and/or oral calcium replacement, with or without vitamin D.

Hypocalcemia following Prolia administration is a significant risk in patients with severe renal impairment (creatinine clearance < 30 mL/min) or receiving dialysis. These patients may also develop marked elevations of serum parathyroid hormone (PTH). Concomitant use of calcimimetic drugs may worsen hypocalcemia risk and serum calcium should be closely monitored. Instruct all patients with severe renal impairment, including those receiving dialysis, about the symptoms of hypocalcemia and the importance of maintaining calcium levels with adequate calcium and vitamin D supplementation.

Adequately supplement all patients with calcium and vitamin D.

Osteonecrosis of the Jaw

Osteonecrosis of the jaw (ONJ), which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing. ONJ has been reported in patients receiving denosumab.

Dental examination is recommended prior to treatment in patients with risk factors for ONJ such as invasive dental procedures (e.g. tooth extraction, dental implants, oral surgery), diagnosis of cancer, concomitant therapies (e.g. chemotherapy, corticosteroids, angiogenesis inhibitors), poor oral hygiene, and comorbid disorders (e.g. periodontal and/or other pre-existing dental disease, anemia, coagulopathy, infection, ill-fitting dentures). Good oral hygiene practices should be maintained during treatment with Prolia. Concomitant administration of drugs associated with ONJ may increase the risk of developing ONJ. The risk of ONJ may increase with duration of exposure to Prolia.

For patients requiring invasive dental procedures, clinical judgment of the treating physician and/or oral surgeon should guide the management plan of each patient based on individual benefit-risk assessment.

Patients who are suspected of having or who develop ONJ while on Prolia should receive care by a dentist or an oral surgeon. In these patients, extensive dental surgery to treat ONJ may exacerbate the condition. Discontinuation of Prolia therapy should be considered based on individual benefit-risk assessment.

Atypical Subtrochanteric and Diaphyseal Femoral Fractures

Atypical low energy or low trauma fractures of the shaft have been reported in patients receiving Prolia. These fractures can occur anywhere in the femoral shaft from just below the lesser trochanter to above the supracondylar flare and are transverse or short oblique in orientation without evidence of comminution. Causality has not been established as these fractures also occur in osteoporotic patients who have not been treated with antiresorptive agents.

Atypical femoral fractures most commonly occur with minimal or no trauma to the affected area. They may be bilateral, and many patients report prodromal pain in the affected area, usually presenting as dull, aching thigh pain, weeks to months before a complete fracture occurs.

Any patient who presents with thigh or groin pain should be suspected of having an atypical fracture and should be evaluated to rule out an incomplete femur fracture. Patient presenting with an atypical femur fracture should also be assessed for symptoms and signs of fracture in the contralateral limb. Interruption of Prolia therapy should be considered, pending a benefit-risk assessment, on an individual basis.

Multiple Vertebral Fractures (MVF) Following Discontinuation of Prolia Treatment

Following discontinuation of Prolia treatment, fracture risk increases, including the risk of multiple vertebral fractures. Treatment with Prolia results in significant suppression of bone turnover and cessation of Prolia treatment results in increased bone turnover above pretreatment values 9 months after the last dose of Prolia. Bone turnover then returns to pretreatment values 24 months after the last dose of Prolia. In addition, bone mineral density returns to pretreatment values within 18 months after the last injection.

New vertebral fractures occurred as early as 7 months (on average 19 months) after the last dose of Prolia. Prior vertebral fracture was a predictor of multiple vertebral fractures after Prolia discontinuation.

Evaluate an individual's benefit-risk before initiating treatment with Prolia.

If Prolia treatment is discontinued, patients should be transitioned to an alternative antiresorptive therapy.

Serious Infections

In a clinical trial of over 7800 women with postmenopausal osteoporosis, serious infections leading to hospitalization were reported more frequently in the Prolia group than in the placebo group. Serious skin infections, as well as infections of the abdomen, urinary tract, and ear, were more frequent in patients treated with Prolia. Endocarditis was also reported more frequently in Prolia-treated patients. The incidence of opportunistic infections was similar between placebo and Prolia groups, and the overall incidence of infections was similar between the treatment groups. Advise patients to seek prompt medical attention if they develop signs or symptoms of severe infection, including cellulitis.

Patients on concomitant immunosuppressant agents or with impaired immune systems may be at increased risk for serious infections. Consider the benefit-risk profile in such patients before treating with Prolia. In patients who develop serious infections while on Prolia, prescribers should assess the need for continued Prolia therapy.

Dermatologic Adverse Reactions

In a large clinical trial of over 7800 women with postmenopausal osteoporosis, epidermal and dermal adverse events such as dermatitis, eczema, and rashes occurred at a significantly higher rate in the Prolia group compared to the placebo group. Most of these events were not specific to the injection site

Musculoskeletal Pain

In postmarketing experience, severe and occasionally incapacitating bone, joint, and/or muscle pain has been reported in patients taking Prolia.

Suppression of Bone Turnover

In clinical trials in women with postmenopausal osteoporosis, treatment with Prolia resulted in significant suppression of bone remodeling as evidenced by markers of bone turnover and bone histomorphometry. The significance of these findings and the effect of long-term treatment with Prolia are unknown. The long-term consequences of the degree of suppression of bone remodeling observed with Prolia may contribute to adverse outcomes such as osteonecrosis of the jaw, atypical fractures, and delayed fracture healing. Monitor patients for these consequences.

ADVERSE REACTIONS

The following serious adverse reactions are discussed below:

- Hypocalcemia
- Serious Infections
- Dermatologic Adverse Reactions
- Osteonecrosis of the Jaw
- Atypical Subtrochanteric and Diaphyseal Femoral Fractures
- Multiple Vertebral Fractures (MVF) Following Discontinuation of Prolia Treatment

The most common adverse reactions reported with Prolia in patients with postmenopausal osteoporosis are back pain, pain in extremity, musculoskeletal pain, hypercholesterolemia, and cystitis.

The most common adverse reactions reported with Prolia in men with osteoporosis are back pain, arthralgia, and nasopharyngitis.

The most common adverse reactions reported with Prolia in patients with glucocorticoid-induced osteoporosis are back pain, hypertension, bronchitis, and headache.

The most common (per patient incidence ≥ 10%) adverse reactions reported with Prolia in patients with bone loss receiving androgen deprivation therapy for prostate cancer or adjuvant aromatase inhibitor therapy for breast cancer are arthralgia and back pain. Pain in extremity and musculoskeletal pain have also been reported in clinical trials.

The most common adverse reactions leading to discontinuation of Prolia in patients with postmenopausal osteoporosis are back pain and constipation.

Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in clinical practice.

Treatment to Increase Bone Mass in Men with Osteoporosis

The safety of Prolia in the treatment of men with osteoporosis was assessed in a 1-year randomized, double-blind, placebo-controlled study. A total of 120 men were exposed to placebo and 120 men were exposed to Prolia administered subcutaneously once every 6 months as a single 60 mg dose. All men were instructed to take at least 1000 mg of calcium and 800 IU of vitamin D supplementation per day.

The incidence of all-cause mortality was 0.8% (n = 1) in the placebo group and 0.8% (n = 1) in the Prolia group. The incidence of nonfatal serious adverse events was 7.5% in the placebo group and 8.3% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 0% and 2.5% for the placebo and Prolia groups, respectively.

Adverse reactions reported in ≥ 5% of men with osteoporosis and more frequently with Prolia than in the placebo-treated patients were: back pain (6.7% placebo vs. 8.3% Prolia), arthralgia (5.8% placebo vs. 6.7% Prolia), and nasopharyngitis (5.8% placebo vs. 6.7% Prolia).

Serious Infections

Serious infection was reported in 1 patient (0.8%) in the placebo group and no patients in the Prolia group.

Dermatologic Adverse Reactions

Epidermal and dermal adverse events (such as dermatitis, eczema, and rashes) were reported in 4 patients (3.3%) in the placebo group and 5 patients (4.2%) in the Prolia group.

Osteonecrosis of the Jaw

No cases of ONJ were reported.

Pancreatitis

Pancreatitis was reported in 1 patient (0.8%) in the placebo group and 1 patient (0.8%) in the Prolia group.

New Malignancies

New malignancies were reported in no patients in the placebo group and 4 (3.3%) patients (3 prostate cancers, 1 basal cell carcinoma) in the Prolia group.

Treatment of Glucocorticoid-Induced Osteoporosis

The safety of Prolia in the treatment of glucocorticoid-induced osteoporosis was assessed in the 1-year, primary analysis of a 2-year randomized, multicenter, double-blind, parallel-group, active-controlled study of 795 patients (30% men and 70% women) aged 20 to 94 (mean age of 63 years) treated with greater than or equal to 7.5 mg/day oral prednisone (or equivalent). A total of 384 patients were exposed to 5 mg oral daily bisphosphonate (active-control) and 394 patients were exposed to Prolia administered once every 6 months as a 60 mg subcutaneous dose. All patients were instructed to take at least 1000 mg of calcium and 800 IU of vitamin D supplementation per day.

The incidence of all-cause mortality was 0.5% (n = 2) in the active-control group and 1.5% (n = 6) in the Prolia group. The incidence of serious adverse events was 17% in the active-control group and 16% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 3.6% and 3.8% for the active-control and Prolia groups, respectively.

Adverse reactions reported in ≥ 2% of patients with glucocorticoid-induced osteoporosis and more frequently with Prolia than in the active-control-treated patients are shown in the table below

Table. Adverse Reactions Occurring in ≥ 2% of Patients with Glucocorticoid-induced Osteoporosis and More Frequently with Prolia than in Active-Control-treated Patients

Preferred Term	Prolia (N = 394) n (%)	Oral Daily Bisphosphonate (Active-Control) (N = 384) n (%)
Back pain	18 (4.6)	17 (4.4)
Hypertension	15 (3.8)	13 (3.4)
Bronchitis	15 (3.8)	11 (2.9)
Headache	14 (3.6)	7 (1.8)
Dyspepsia	12 (3.0)	10 (2.6)
Urinary tract infection	12 (3.0)	8 (2.1)

Abdominal pain upper	12 (3.0)	7 (1.8)
Upper respiratory tract infection	11 (2.8)	10 (2.6)
Constipation	11 (2.8)	6 (1.6)
Vomiting	10 (2.5)	6 (1.6)
Dizziness	9 (2.3)	8 (2.1)
Fall	8 (2.0)	7 (1.8)
Polymyalgia rheumatica*	8 (2.0)	1 (0.3)

*Events of worsening of underlying polymyalgia rheumatica.

Osteonecrosis of the Jaw
No cases of ONJ were reported.

Atypical Subtrochanteric and Diaphyseal Femoral Fractures

Atypical femoral fractures were reported in 1 patient treated with Prolia. The duration of Prolia exposure to time of atypical femoral fracture diagnosis was at 8.0 months.

Serious Infections

Serious infection was reported in 15 patients (3.9%) in the active-control group and 17 patients (4.3%) in the Prolia group.

Dermatologic Adverse Reactions

Epidermal and dermal adverse events (such as dermatitis, eczema, and rashes) were reported in 16 patients (4.2%) in the active-control group and 15 patients (3.8%) in the Prolia group.

Treatment of Bone Loss in Patients Receiving Androgen Deprivation Therapy for Prostate Cancer or Adjuvant Aromatase Inhibitor Therapy for Breast Cancer

The safety of Prolia in the treatment of bone loss in men with nonmetastatic prostate cancer receiving androgen deprivation therapy (ADT) was assessed in a 3-year, randomized, double-blind, placebo-controlled, multinational study of 1468 men aged 48 to 97 years. A total of 725 men were exposed to placebo and 731 men were exposed to Prolia administered once every 6 months as a single 60 mg subcutaneous dose. All men were instructed to take at least 1000 mg of calcium and 400 IU of vitamin D supplementation per day.

The incidence of serious adverse events was 30.6% in the placebo group and 34.6% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 6.1% and 7.0% for the placebo and Prolia groups, respectively.

The safety of Prolia in the treatment of bone loss in women with nonmetastatic breast cancer receiving aromatase inhibitor (AI) therapy was assessed in a 2-year, randomized, double-blind, placebo-controlled, multinational study of 252 postmenopausal women aged 35 to 84 years. A total of 120 women were exposed to placebo and 129 women were exposed to Prolia administered once every 6 months as a single 60 mg subcutaneous dose. All women were instructed to take at least 1000 mg of calcium and 400 IU of vitamin D supplementation per day.

The incidence of serious adverse events was 9.2% in the placebo group and 14.7% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 4.2% and 0.8% for the placebo and Prolia groups, respectively.

Adverse reactions reported in ≥ 10% of Prolia-treated patients receiving ADT for prostate cancer or adjuvant AI therapy for breast cancer, and more frequently than in the placebo-treated patients were: arthralgia (13.0% placebo vs. 14.3% Prolia) and back pain (10.5% placebo vs. 11.5% Prolia). Pain in extremity (7.7% placebo vs. 9.9% Prolia) and musculoskeletal pain (3.8% placebo vs. 6.0% Prolia) have also been reported in clinical trials. Additionally, in Prolia-treated men with nonmetastatic prostate cancer receiving ADT, a greater incidence of cataracts was observed (1.2% placebo vs. 4.7% Prolia).

Hypocalcemia (serum calcium < 8.4 mg/dL) was reported only in Prolia-treated patients (2.4% vs. 0.0%) at the month 1 visit.

Immunogenicity

Denosumab is a human monoclonal antibody. As with all therapeutic proteins, there is potential for immunogenicity. Using an electrochemiluminescent bridging immunoassay, less than 1% (55 out of 8113) of patients treated with Prolia for up to 5 years tested positive for binding antibodies (including pre-existing, transient, and developing antibodies). None of the patients tested positive for neutralizing antibodies, as was assessed using a chemiluminescent cell-based in vitro biological assay. No evidence of altered pharmacokinetic profile, toxicity profile, or clinical response was associated with binding antibody development.

The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of a positive antibody (including neutralizing antibody) test result may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of antibodies to denosumab with the incidence of antibodies to other products may be misleading.

6.3. Non-clinical Data of Innovator Denosumab

Carcinogenicity

The carcinogenic potential of denosumab has not been evaluated in long-term animal studies.

Mutagenicity

The genotoxic potential of denosumab has not been evaluated.

Impairment of Fertility

Denosumab had no effect on female fertility or male reproductive organs in monkeys at doses that were 13- to 50-fold higher than the recommended human dose of 60 mg subcutaneously administered once every 6 months, based on body weight (mg/kg).

Animal Toxicology and/or Pharmacology

Denosumab is an inhibitor of osteoclastic bone resorption via inhibition of RANKL.

In ovariectomized monkeys, once-monthly treatment with denosumab suppressed bone turnover and increased bone mineral density (BMD) and strength of cancellous and cortical bone at doses 50-fold higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg). Bone tissue was normal with no evidence of mineralization defects, accumulation of osteoid, or woven bone.

Because the biological activity of denosumab in animals is specific to nonhuman primates, evaluation of genetically engineered ("knockout") mice or use of other biological inhibitors of the RANK/RANKL pathway, namely OPG-Fc, provided additional information on the pharmacodynamic properties of denosumab. RANK/RANKL knockout mice exhibited absence of lymph node formation, as well as an absence of lactation due to inhibition of mammary gland maturation (lobulo-alveolar gland development during pregnancy). Neonatal RANK/RANKL knockout mice exhibited reduced bone growth and lack of tooth eruption. A corroborative study in 2-week-old rats given the RANKL inhibitor OPG-Fc also showed reduced bone growth, altered growth plates, and impaired tooth eruption. These changes were partially reversible in this model when dosing with the RANKL inhibitors was discontinued.

6.4. Clinical Data of Innovator Denosumab

Treatment to Increase Bone Mass in Men with Osteoporosis

The efficacy and safety of Prolia in the treatment to increase bone mass in men with osteoporosis was demonstrated in a 1-year, randomized, double-blind, placebo-controlled trial. Enrolled men had a baseline BMD T-score between -2.0 and -3.5 at the lumbar spine or femoral neck. Men with a BMD T-score

between -1.0 and -3.5 at the lumbar spine or femoral neck were also enrolled if there was a history of prior fragility fracture. Men with other diseases (such as rheumatoid arthritis, osteogenesis imperfecta, and Paget's disease) or on therapies that may affect bone were excluded from this study. The 242 men enrolled in the study ranged in age from 31 to 84 years with a mean age of 65 years. Men were randomized to receive SC injections of either placebo (n = 121) or Prolia 60 mg (n = 121) once every 6 months. All men received at least 1000 mg calcium and at least 800 IU vitamin D supplementation daily.

Effect on Bone Mineral Density (BMD)

The primary efficacy variable was percent change in lumbar spine BMD from baseline to 1-year. Secondary efficacy variables included percent change in total hip, and femoral neck BMD from baseline to 1-year.

Treatment with Prolia significantly increased BMD at 1-year. The treatment differences in BMD at 1-year were 4.8% (+0.9% placebo, +5.7% Prolia; (95% CI: 4.0, 5.6); $p < 0.0001$) at the lumbar spine, 2.0% (+0.3% placebo, +2.4% Prolia) at the total hip, and 2.2% (0.0% placebo, +2.1% Prolia) at femoral neck. Consistent effects on BMD were observed at the lumbar spine regardless of baseline age, race, BMD, testosterone concentrations, and level of bone turnover.

Bone Histology and Histomorphometry

A total of 29 transiliac crest bone biopsy specimens were obtained from men with osteoporosis at 12 months (17 specimens in Prolia group, 12 specimens in placebo group). Of the biopsies obtained, 29 (100%) were adequate for qualitative histology and, in Prolia patients, 6 (35%) were adequate for full quantitative histomorphometry assessment. Qualitative histology assessments showed normal architecture and quality with no evidence of mineralization defects, woven bone, or marrow fibrosis in patients treated with Prolia. The presence of double tetracycline labeling in a biopsy specimen provides an indication of active bone remodeling, while the absence of tetracycline label suggests suppressed bone formation. In patients treated with Prolia, 6% had no tetracycline label present at the month 12 biopsy, while 100% of placebo-treated patients had double label present. When compared to placebo, treatment with Prolia resulted in markedly reduced bone formation rates. However, the long-term consequences of this degree of suppression of bone remodeling are unknown.

Treatment of Glucocorticoid-Induced Osteoporosis

The efficacy and safety of Prolia in the treatment of patients with glucocorticoid-induced osteoporosis was assessed in the 12-month primary analysis of a 2-year, randomized, multicenter, double-blind, parallel-group, active-controlled study (NCT 01575873) of 795 patients (70% women and 30% men) aged 20 to 94 years (mean age of 63 years) treated with greater than or equal to 7.5 mg/day oral prednisone (or

equivalent) for < 3 months prior to study enrollment and planning to continue treatment for a total of at least 6 months (glucocorticoid-initiating subpopulation; n = 290) or ≥ 3 months prior to study enrollment and planning to continue treatment for a total of at least 6 months (glucocorticoid-continuing subpopulation, n = 505). Enrolled patients < 50 years of age were required to have a history of osteoporotic fracture. Enrolled patients ≥ 50 years of age who were in the glucocorticoid-continuing subpopulation were required to have a baseline BMD T-score of ≤ -2.0 at the lumbar spine, total hip, or femoral neck; or a BMD T-score ≤ -1.0 at the lumbar spine, total hip, or femoral neck and a history of osteoporotic fracture.

Patients were randomized (1:1) to receive either an oral daily bisphosphonate (active-control, risedronate 5 mg once daily) (n = 397) or Prolia 60 mg subcutaneously once every 6 months (n = 398) for one year. Randomization was stratified by gender within each subpopulation. Patients received at least 1000 mg calcium and 800 IU vitamin D supplementation daily.

Effect on Bone Mineral Density (BMD)

In the glucocorticoid-initiating subpopulation, Prolia significantly increased lumbar spine BMD compared to the active-control at one year (Active-control 0.8%, Prolia 3.8%) with a treatment difference of 2.9% ($p < 0.001$). In the glucocorticoid-continuing subpopulation, Prolia significantly increased lumbar spine BMD compared to active-control at one year (Active-control 2.3%, Prolia 4.4%) with a treatment difference of 2.2% ($p < 0.001$). Consistent effects on lumbar spine BMD were observed regardless of gender; race; geographic region; menopausal status; and baseline age, lumbar spine BMD T-score, and glucocorticoid dose within each subpopulation.

Bone Histology

Bone biopsy specimens were obtained from 17 patients (11 in the active-control treatment group and 6 in the Prolia treatment group) at Month 12. Of the biopsies obtained, 17 (100%) were adequate for qualitative histology. Qualitative assessments showed bone of normal architecture and quality without mineralization defects or bone marrow abnormality. The presence of double tetracycline labeling in a biopsy specimen provides an indication of active bone remodeling, while the absence of tetracycline label suggests suppressed bone formation. In patients treated with active-control, 100% of biopsies had tetracycline label. In patients treated with Prolia, 1 (33%) had tetracycline label and 2 (67%) had no tetracycline label present at the 12-month biopsy. Evaluation of full quantitative histomorphometry including bone remodeling rates was not possible in the glucocorticoid-induced osteoporosis population treated with

Prolia. The long-term consequences of this degree of suppression of bone remodeling in glucocorticoid-treated patients is unknown.

Treatment of Bone Loss in Men with Prostate Cancer

The efficacy and safety of Prolia in the treatment of bone loss in men with nonmetastatic prostate cancer receiving androgen deprivation therapy (ADT) were demonstrated in a 3-year, randomized (1:1), double-blind, placebo-controlled, multinational study. Men less than 70 years of age had either a BMD T-score at the lumbar spine, total hip, or femoral neck between -1.0 and -4.0, or a history of an osteoporotic fracture. The mean baseline lumbar spine BMD T-score was -0.4, and 22% of men had a vertebral fracture at baseline. The 1468 men enrolled ranged in age from 48 to 97 years (median 76 years). Men were randomized to receive subcutaneous injections of either placebo (n = 734) or Prolia 60 mg (n = 734) once every 6 months for a total of 6 doses. Randomization was stratified by age (< 70 years vs. ≥ 70 years) and duration of ADT at trial entry (≤ 6 months vs. > 6 months). Seventy-nine percent of patients received ADT for more than 6 months at study entry. All men received at least 1000 mg calcium and 400 IU vitamin D supplementation daily.

Effect on Bone Mineral Density (BMD)

The primary efficacy variable was percent change in lumbar spine BMD from baseline to month 24. An additional key secondary efficacy variable was the incidence of new vertebral fracture through month 36 diagnosed based on x-ray evaluation by two independent radiologists. Lumbar spine BMD was higher at 2 years in Prolia-treated patients as compared to placebo-treated patients [-1.0% placebo, +5.6% Prolia; treatment difference 6.7% (95% CI: 6.2, 7.1); p < 0.0001].

With approximately 62% of patients followed for 3 years, treatment differences in BMD at 3 years were 7.9% (-1.2% placebo, +6.8% Prolia) at the lumbar spine, 5.7% (-2.6% placebo, +3.2% Prolia) at the total hip, and 4.9% (-1.8% placebo, +3.0% Prolia) at the femoral neck. Consistent effects on BMD were observed at the lumbar spine in relevant subgroups defined by baseline age, BMD, and baseline history of vertebral fracture.

Effect on Vertebral Fractures

Prolia significantly reduced the incidence of new vertebral fractures at 3 years (p = 0.0125), as shown in Table.

Table. The Effect of Prolia on the Incidence of New Vertebral Fractures in Men with Nonmetastatic Prostate Cancer

	Proportion of Men with Fracture (%)+		Absolute Risk Reduction (%)* (95% CI)	Relative Risk Reduction (%)* (95% CI)
	Placebo N = 673 (%)	Prolia N = 679 (%)		
0-1 Year	1.9	0.3	1.6 (0.5, 2.8)	85 (33, 97)
0-2 Years	3.3	1.0	2.2 (0.7, 3.8)	69 (27, 86)
0-3 Years	3.9	1.5	2.4 (0.7, 4.1)	62 (22, 81)

+ Event rates based on crude rates in each interval.

* Absolute risk reduction and relative risk reduction based on Mantel-Haenszel method adjusting for age group and ADT duration variables.

6.5. Rationale for the Study:

Sponsor has developed Biosimilar Denosumab (ENZ215). ENZ215, a proposed biosimilar to Prolia, is a monoclonal antibody which is structurally and functionally similar to Prolia. This study will investigate the similarity of ENZ215 of Enzene Biosciences Ltd. with EU-sourced Prolia®, and US-sourced Prolia®. This study aims to confirm bioequivalence of ENZ215 to Prolia in terms of pharmacokinetics, pharmacodynamics, safety, tolerability, and immunogenicity in healthy adult male volunteers.

Healthy male subjects are selected for the study as they lack comorbidities, and are not on medications, are not immunocompromised, and therefore represent the most sensitive population to detect differences in PK between the test and reference products.

7. STUDY OBJECTIVES AND ENDPOINTS

7.1. STUDY OBJECTIVES

Primary objective:

- To demonstrate bioequivalence between ENZ215 and EU- and US-sourced Prolia® using PK parameters

Secondary Objective(s):

- To compare the serum PK profile of ENZ215 and EU- and US-sourced Prolia®
- To compare the serum CTX-1 profile of ENZ215 and EU- and US-sourced Prolia®
- To compare the immunogenicity profile of ENZ215 and EU- and US-sourced Prolia®
- To compare the safety and tolerability profile of ENZ215 and EU- and US-sourced Prolia®

7.2. STUDY ENDPOINTS

Primary endpoint:

- Maximum observed drug concentration (C_{max}), area under the drug concentration-time curve from time 0 to infinity (AUC_{0-inf}) and area under the drug concentration-time curve from day 0 to day 270 (AUC_{0-t}) of ENZ215 and EU- and US-sourced Prolia®

Secondary endpoint(s) (Pharmacokinetics):

- Partial area under the drug concentration-time curve from time 0 (pre-dose) to day 28
- Time to reach C_{max} (t_{max})
- Terminal elimination half-life ($t_{1/2}$)
- Apparent systemic clearance (CL/F)

Secondary endpoint(s) (Pharmacodynamics):

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

Safety endpoint(s):

- 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.

8. INVESTIGATIONAL PLAN

8.1. STUDY DURATION AND DESIGN

This is a randomized, double-blind, three-arm, parallel-group, single-dose study in healthy adult male volunteers.

Approximately 207 subjects will be enrolled into 3 groups (69 in each group) in parallel. The subjects may be enrolled in multiple groups at the site. All eligible subjects will be assigned to one of the three treatment groups in 1:1:1 ratio i.e. ENZ215 or US-sourced Prolia® or EU-sourced Prolia® to enter into the study period of 39 weeks. The study duration will be approximately 16 months (i.e. 6 months of recruitment period, 4 weeks of screening period and approximately 39 weeks (270 days) of study period).

Each subject will be required to visit the site for a total of 20 visits: visit 1 – screening visit, visit 2 – day 0 to day 2, visit 3 – day 3, visit 4 – day 4, visit 5 – day 5, visit 6 – day 6, visit 7 – day 8, visit 8 – day 10, visit 9 – day 12, visit 10 – day 16, visit 11 – day 21, visit 12 – day 28 (week 4), visit 13 – day 42 (week 6), visit 14 – day 63 (week 9), visit 15 – day 90 (week 13), visit 16 – day 119 (week 17), visit 17 – day 147 (week 21), visit 18 – day 180 (week 26), visit 19 day – 224 (week 32), and visit 20 – day 270 (week 39). A window period of ±1 day is allowed for visit 12 (day 28), window period of ±3 days are allowed from day 42 (week 6) to day 180 (week 26), A window period of ±5 days are allowed from day 224 (week 32) to day 270 (week 39).

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

During the study, blood and urine samples will be collected for eligibility and safety (hematology, serology, routine serum chemistry, serum chemistry (calcium only), urine analysis, Anti-Denosumab antibody and neutralizing antibody), PK analysis and serum CTX-1 assessments.

8.2. RANDOMIZATION AND BLINDING

RANDOMIZATION

The selection of Test (A), Reference (B) or Reference (C) products for each subject during the study will be determined according to the randomization schedule. The randomization will be generated by statistician in a 1:1:1 ratio of treatment allotment for test and reference products, respectively. The randomization schedule will be generated by the statistician.

A total of 207 subjects will be randomized to either of the three treatment groups:

- Group A (N = 69): ENZ215 (Biosimilar Denosumab)
- Group B (N = 69): Innovator Denosumab (US-sourced Prolia®)
- Group C (N = 69): Innovator Denosumab (EU-sourced Prolia®)

Subjects who fulfill the eligibility criteria will be assigned a randomization code.

BLINDING

This study is a randomized, double-blind study. The blinding details will be detailed in pharmacy manual and/or unblinding plan.

METHOD OF TREATMENT ASSIGNMENT

Each subject will be assigned a unique identification number upon screening. Subjects who complete the study screening assessments and meet all the eligibility criteria will be assigned a unique randomization

identification number at the time of the dosing, different from the screening number, and will receive the corresponding study drug.

Subjects will 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.

MAINTENANCE OF RANDOMIZATION

A computerized randomization scheme will be created by a statistician and it shall be considered blinded (as per the following).

The randomization will be made available only to the clinic pharmacy staff preparing the drug who will not be involved in any other aspect of the study including administration of the drug.

PROCEDURES FOR BREAKING THE BLIND PRIOR TO STUDY COMPLETION

Breaking of the blind is 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.

In the event of a medical emergency, it is requested that the PI or designee make every effort to contact the Study Monitor or designee prior to breaking the blind. If breaking the blind is required because of a medical emergency, the treatment identity would be revealed by the PI or designee, for that subject only. In the event that the emergency is one, in which it appears that the other subjects may be at imminent risk, the blind may be broken for all subjects dosed. The unblinding will be properly documented in the study file.

In all cases where the code is broken, the PI or designee should record the date and reason for code breaking.

At the end of the study, envelopes will be retained according to site procedures.

9. POPULATION

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 will be enrolled in this study.

9.1. ENTRY CRITERIA

The Investigator must ensure that all subjects being considered for the study meet the following (all the inclusion and none of the exclusion) criteria. A subjects' selection will be established by reviewing all inclusion/exclusion criteria at screening and baseline.

9.1.1. INCLUSION CRITERIA

The subjects will be included in the study based on the following 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/m^2 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: A 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).

9.1.2. EXCLUSION CRITERIA

The subjects will be excluded from the study based on the following 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).

9.2. DISCONTINUATION/WITHDRAWAL CRITERIA

The subject may be withdrawn/discontinued from the study due to the following reasons:

1. The subject suffers from significant inter-current illness or undergoes surgery during the course of the study
2. Subject's voluntary withdrawal of consent
3. Subject non-compliance to pre- and post-dose requirements
4. Protocol violation
5. In Investigator's opinion it is not in the subject's best interest to continue
6. The subject is found to conceal important medical history which in the opinion of Investigator may compromise his safety during participation in this study
7. Subjects experiencing signs or symptoms of systemic hypersensitivity reactions, anaphylactic or other clinically significant allergic reactions during the study
8. Any AE or SAE which requires discontinuation of subject in opinion of Investigator
9. Study termination by the Sponsor
10. Any other justifiable reason, which should be adequately documented

9.3. DATA COLLECTION AND FOLLOW-UP FOR DISCONTINUED SUBJECTS

Investigator should try to obtain the reason from subject while withdrawing consent. Reason for withdrawal from the study shall be documented, whenever possible.

EOS assessment (As per Section 10.5) shall be performed for all prematurely discontinued and withdrawn subjects. For all discontinued subjects, data collected till the time of discontinuation shall be reported in (e)CRF.

Safety data shall be collected for all discontinued subjects, who are discontinued due to an adverse event (AE) or serious adverse event (SAE). In any case, every effort must be made to undertake protocol-specified safety follow-up procedures. If the subject is discontinued due to an event, subject should be given an appropriate care under medical supervision until the symptoms of any AE is resolved or the subject's condition becomes stable.

9.4. REPLACEMENT OF SUBJECTS

10 % drop-out rate has been considered while estimating the sample size, hence subjects who withdraw or are withdrawn from the study will not be replaced.

9.5. STUDY TREATMENTS

A Separate IP management plan will be prepared to describe procedure for handling of IPs.

9.5.1. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

Clinical supplies (test and reference drug products) will be packed, labeled, handled and stored in accordance with current Good Manufacturing Practice of investigational products.

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

9.6. INVESTIGATIONAL PRODUCT ADMINISTRATION

Based on the randomization schedule, investigational product (either ENZ215 or EU- or US-sourced Prolia) will be administered to the subjects at a dose of 60 mg (single-dose) subcutaneously on Day 1 into the upper thigh.

Special precautions for disposal and handling

- Before administration, the solution should be inspected. Do not inject the solution if it contains particles, or is cloudy or discoloured.
- Do not shake.
- To avoid discomfort at the site of injection, allow the PFS to reach room temperature (up to 25°C) before injecting and inject slowly. This generally takes 15 to 30 minutes. Do not warm investigational product in any other way.
- Inject the entire content of the pre-filled syringe.

9.7. INVESTIGATIONAL PRODUCT RECEIPT AND STORAGE

Investigational products (test and reference products) will be supplied to CRO or trial site in sufficient quantities as required by the study,. The received investigational products will be verified with Certificates of analysis (COA)/packaging list, for the sealed condition of packs and adequacy of the label, including product name, strength, number of dosage units, lot number or batch number, expiry date/retest date and storage condition mentioned clearly.

It is the Investigator's or Investigator's designated personnel (such as pharmacist) responsibility to perform the accountability check and verify that the shipment contains all the items noted in the shipment inventory including a check for any damages or unusable investigational product. A record of the inventory received should be documented in the drug accountability form. The Investigator or the Investigator's designated personnel, should verify the investigational product, and sign and date the drug receipt and maintain a copy of the receipt at the study center.

Investigational product labels will be prepared in compliance to the applicable regulatory requirements and in house procedure. The labels should contain at least the below mentioned details.

- Name of the Sponsor
- Study protocol number
- Randomization ID
- Subject details
- Lot/batch number
- Dosing instructions
- Storage conditions
- Manufacturing date
- Expiry/Retest date
- Statement: 'For clinical trial use only"

Temperature will be monitored on a daily basis during the conduct of the study. The investigational products will be stored as per the product requirements.

The Investigator, site pharmacist or other Investigator's assigned personnel allowed to receive, store, issue and dispense the investigational products will be responsible for ensuring that the investigational products to be used in the clinical trial is securely maintained as specified by the Sponsor and in accordance with the current Good Clinical Practice.

All investigational products shall be issued and used in accordance with the protocol and it is the Investigator's responsibility to ensure that an accurate record of the investigational products issued and returned is maintained.

Under no circumstances will the Investigator supply the investigational product to a third party, allow the investigational product to be used other than as directed by this clinical trial protocol, or dispose off the investigational product in any other manner.

9.8. ACCOUNTABILITY OF INVESTIGATIONAL PRODUCTS

The Investigator or Investigator's designated personnel will maintain a careful record of the inventory and disposition of all agents received for study using a drug accountability form or log. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received
- Amount currently in place
- Label ID number or batch number and use by date or expiry date
- Dates and initials of person responsible for investigational product inventory
- Amount dispensed, including unique subject identifiers
- Non-study disposition (e.g., lost, wasted, broken)
- Amount returned to Sponsor
- Amount destroyed at study site, if applicable

9.9. RETRIEVAL OF INVESTIGATIONAL PRODUCTS

IP (test or reference product) will be administered to the subject on day 1.

At the completion of the study, there will be a final reconciliation of investigational product. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused investigational product.

9.10. UNUSED INVESTIGATIONAL PRODUCTS

Units of investigational products that have not been dispensed will be retained in their original containers. Any product that had been dispensed but not used will be labeled as '**UNUSED'** and returned to Sponsor/Sponsor designee or destroyed at site as applicable. Retention sample shall be retained for at least 05 years following the date of completion of a clinical stage of the study.

Note: *If investigational products are to be destroyed on site, it is the Investigator's responsibility to ensure that arrangements have been made for disposal and written authorization has been granted by the Sponsor, and the procedures for proper disposal have been established according to applicable regulations, guidelines, Sponsor's instructions and institutional procedures. Appropriate records of the disposal must be maintained. The unused study products can only be destroyed after being inspected and reconciled by the responsible study monitor.*

10. STUDY PROCEDURES

The study procedures described below will be carried out at the study visits.

10.1. Visit 1 / Screening (Day -28 to Day -1)

The screening procedures will be completed within 28 days (4 weeks) from the signing of the informed consent form (ICF).

- Written informed consent will be taken from the subjects. No trial related procedure will be conducted prior to obtaining informed consent.
- Assessment of subject eligibility as per inclusion and exclusion criteria
- Demographic parameters (age, BMI, body weight [in kg], height, race, ethnicity) will be noted
- Medical/Surgical history - relevant medical history will be collected including prior and ongoing medical illnesses, conditions, and surgical procedures
- Prior medication history
- Physical examination
- Oral examination and recommendation for appropriate preventive dentistry if applicable
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- COVID-19 test - COVID testing will be performed in line with the clinical site's current COVID prevention of infection management strategy and applicable local government/health guidelines
- 12-lead ECG
- Blood sample collection for the following;

- ✓ Hematology- Hb, platelet count, RBC count, WBC count, differential (absolute and percentage) WBC count
- ✓ Serology- HIV, HBsAg, HCV
- ✓ Serum chemistry- liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], total bilirubin, albumin and total proteins, kidney function tests (blood urea nitrogen [BUN[@]], creatinine), electrolytes (sodium, potassium, chloride), glucose, serum calcium, albumin-adjusted calcium, magnesium, and phosphorus.

- Urine sample collection for routine urine analysis: Color, transparency, pH, specific gravity, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, leucocyte esterase, and protein; Microscopic examination: RBC, leucocytes, epithelial cells and bacteria, crystals.

- AE/SAE recording and reporting
- Concomitant medications assessment
- Urine screen for drugs of abuse (as a local standard : benzodiazepines, barbiturates, amphetamines, cocaine, cannabinoids, Morphine)
- Urine screen for cotinine test
- Alcohol test (breath analyzer or any suitable test)

[@]Note: BUN will be reported in the form of Urea in mmol/L.

10.2. Visit 2 (Day 0 – Day 2):

Day 0: Check-In

- Assessment of subject eligibility as per inclusion and exclusion criteria
- Admission to phase-1 study site
- Physical examination at the time of check-in
- COVID-19 test - COVID testing will be performed in line with the clinical site's current COVID prevention of infection management strategy and applicable local government/health guidelines
- Signs and symptoms of COVID-19 (especially fever, cough, difficulty in breathing, loss of taste and smell). Subjects having signs and symptoms of COVID-19 will be further evaluated for SARS-CoV-2 infection as per the local guidelines at the time of check-in.
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- Alcohol test (breath analyzer or any suitable test)
- Urine screen for drugs of abuse
- Urine screen for cotinine test
- Oral examination and recommendation for appropriate preventive dentistry if applicable
-

- AE/SAE recording and reporting
- Concomitant medication assessment
- Randomization after confirming of all eligibility criteria

Day 1:

- COVID-19 assessment: Signs and Symptoms of COVID-19 (especially fever, cough, difficulty in breathing).
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- Subjects will be randomized if not randomized at Day 0
- IP will be administered as per Section 9.6 under monitoring of Independent study personnel.
- Injection site will be assessed for local reaction post-administration
- Blood sample will be collected for serum chemistry (albumin-adjusted calcium only (pre-dose))
- Blood sample will be collected for Pharmacokinetic analysis at 0 hour (pre-dose), and 1, 4, 8, and 12 hours
- Blood sample will be collected for Pharmacodynamics assessment at 0 hour (pre-dose)
- Blood sample will be collected for Immunogenicity assessment at 0 hour (pre-dose)
- AE/SAE recording and reporting
- Concomitant medication assessment

Day 2: Check-Out

- Physical examination at the time of discharge/check out
- COVID-19 assessment: Signs and Symptoms of COVID-19 (especially fever, cough, difficulty in breathing).
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- Blood sample will be collected for serum chemistry (albumin-adjusted calcium only)
- Blood sample will be collected for Pharmacokinetic analysis at 24 hours
- Blood sample will be collected for Pharmacodynamic assessment
- AE/SAE recording and reporting
- Concomitant medication assessment
- Subjects will be discharged/check-out on day 2 after 24 hour PK and CTX-1 sample collection

10.3. Visit 3 – day 3, visit 4 – day 4, visit 5 – day 5, visit 6 –day 6, visit 7 – day 8, visit 8 – day 10, visit 9 – day 12, visit 10 – day 16 and visit 11 –day 21

- COVID-19 assessment: Signs and Symptoms of COVID-19 (especially fever, cough, difficulty in breathing).
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- Blood sample will be collected for serum chemistry (albumin-adjusted calcium only)
- Blood sample will be collected for Pharmacokinetic analysis
- Blood sample will be collected for Pharmacodynamic assessment
- Blood sample will be collected for Immunogenicity assessment on day 8 and 16
- Urine screen for cotinine test on day 12
- AE/SAE recording and reporting
- Concomitant medication assessment
- Alcohol test (breath analyzer or any suitable test)
  Note: For subjects with COVID-19 symptoms, blood samples can be collected from subjects at their place by third party appointed by CRO/ Sponsor.

10.4. Visit 12 – day 28 (week 4), visit 13 – day 42 (week 6), visit 14 – day 63 (week 9), visit 15 – day 90 (week 13), visit 16 – day 119 (week 17), visit 17 – day 147 (week 21), visit 18 – day 180 (week 26) and visit 19 day – 224 (week 32)

- Note: A window period of ±1 day is allowed for visit 12 (day 28), A window period of ±3 days are allowed from day 42 (week 6) to day 180 (week 26). A window period of ±5 days are allowed on day 224 (week 32) COVID-19 assessment: Signs and Symptoms of COVID-19 (especially fever, cough, difficulty in breathing).
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- Blood sample will be collected for serum chemistry (albumin-adjusted calcium only) on day 180 (week 26)
- Blood sample will be collected for Pharmacokinetic analysis
- Blood sample will be collected for Pharmacodynamic assessment on day 28 (week 4), day 63 (week 9), day 119 (week 17) and day 180 (week 26)
- Blood sample will be collected for Immunogenicity assessment on day 28 (648 hours) (week 4), day 63 (1488 hours) (week 9), day 90 (2136 hours) (week 13), day 119 (2832 hours) (week 17), day 147 (3504 hours) (week 21) and day 180 (4296 hours) (week 26)
- Urine screen for cotinine test
- AE/SAE recording and reporting

- Concomitant medications assessment
- Oral examination and recommendation for appropriate preventive dentistry if applicable
- (on week (s) 4, 13 and 26)
- Alcohol test (breath analyzer or any suitable test)
  Note: For subjects with COVID-19 symptoms, blood samples can be collected from subjects at their place by third party appointed by CRO/ Sponsor.

10.5. Visit 20 – day 270 (week 39)/End of Study Assessment (± 5 days)

End of study assessment will be performed on day 270 (week 39) or at the time of early discontinuation/study withdrawal.

- Physical examination
- COVID-19 assessment: Signs and Symptoms of COVID-19 (especially fever, cough, difficulty in breathing).
- Vital signs will be recorded in supine position after 5 minutes of rest: pulse rate (beats/minute), blood pressure measurement (mm of Hg), respiratory rate (breaths/minute), and body temperature (°C).
- 12-lead ECG
- Blood sample collection for the following;
  - ✓ Hematology- Hb, platelet count, RBC count, WBC count and differential (absolute and percentage) WBC count
  - ✓ Serum chemistry- liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], total bilirubin, albumin and total proteins), kidney function tests (blood urea nitrogen [BUN[@]], creatinine), electrolytes (sodium, potassium, chloride), glucose, serum calcium, albumin-adjusted calcium, magnesium, and phosphorus.
- Urine sample collection for routine urine analysis: color, transparency, pH, specific gravity, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, leucocyte esterase and protein; Microscopic examination: RBC, leucocytes, epithelial cells and bacteria, crystals
- Urine screen for cotinine test
- Blood sample will be collected for Pharmacokinetic analysis
- Blood sample will be collected for Pharmacodynamics assessment
- Blood sample will be collected for Immunogenicity assessment
- AE/SAE recording and reporting
- Concomitant medications assessment
- Oral examination and recommendation for appropriate preventive dentistry if applicable
-
- Alcohol test (breath analyzer or any suitable test)

[@]Note: BUN will be reported in the form of Urea in mmol/L.

Note: For subjects with COVID-19 symptoms, blood samples can be collected from subjects at their place by third party appointed by CRO/ Sponsor.

10.6. Unscheduled Visit

During the study, subjects will be free to contact the Investigator. Subject can come for Unscheduled Visit anytime during the study duration in case of AEs or aggravation of the existing disorder. During an Unscheduled Visit, laboratory tests and other investigations, if required, will be done at the Investigator's discretion.

10.7. PHARMACOKINETIC BLOOD SAMPLING

A total of twenty four (24) blood PK samples of 2.5 mL each will be collected from each subject in the study. Blood samples for PK analysis will be collected at 0 hour (pre-dose), and at 1, 4, 8, and 12 hours (day 1), day 2 (24 hours), day 3 (48 hours), day 4 (72 hours), day 5 (96 hours), day 6 (120 hours), day 8 (168 hours), day 10 (216 hours), day 12 (264 hours), day 16 (360 hours), day 21 (480 hours), day 28 (648 hours) (week 4), day 42 (984 hours) (week 6), day 63 (1488 hours) (week 9), day 90 (2136 hours) (week 13), day 119 (2832 hours) (week 17), day 147 (3504 hours) (week 21), day 180 (4296 hours) (week 26), day 224 (5352 hours) (week 32) and at the end of study (day 270 (6456 hours) (week 39) post-dose).

Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose samples up to day 2 will be collected within ± 10 minutes, within ± 2 hour from day 3 to day 21, within ± 1 day on day 28, within ± 3 days from day 42 to day 180 and within ±5 days from day 224 to day 270

10.8. PHARMACODYNAMIC BLOOD SAMPLING

A total of sixteen (16) blood for serum CTX-1 estimation of 3.5 mL each will be collected from each subject in the study. Blood samples for serum CTX-1 assessment will be collected at 0 hour (pre-dose), and at day 2 (24 hours), day 3 (48 hours), day 4 (72 hours), day 5 (96 hours), day 6 (120 hours), day 8 (168 hours), day 10 (216 hours), day 12 (264 hours), day 16 (360 hours), day 21 (480 hours), day 28 (648 hours) (week 4), day 63 (1488 hours) (week 9), day 119 (2832 hours) (week 17), day 180 (4296 hours) (week 26) and at the end of study (day 270 (6456 hours) (week 39) post-dose).

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. Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose samples up to day 2 will be collected within ± 10 minutes, within ±

2 hour from day 3 to day 21, within ±1 day on day 28, within ± 3 days from day 42 to day 180 and within ±5 days from day 224 to day 270

10.9. IMMUNOGENICITY ASSESSMENT

A total of ten (10) blood Immunogenicity assessment samples of 5.0 mL for NAB (Neutralizing antibodies) and ADA (Anti-Denosumab antibody) will be collected from each subject in the study. Blood samples for Immunogenicity assessment will be collected at 0 hour (pre-dose), day 8 (168 hours), day 16 (360 hours), day 28 (648 hours) (week 4), day 63 (1488 hours) (week 9), day 90 (2136 hours) (week 13), day 119 (2832 hours) (week 17), day 147 (3504 hours) (week 21), day 180 (4296 hours) (week 26) and at the end of study (day 270 (6456 hours) (week 39) post-dose).

Pre-dose sample will be collected within 30 minutes prior to IP administration. Post-dose samples up to day 2 will be collected within ± 10 minutes, within ± 2 hour from day 3 to day 21, within ± 1 day on day 28, within ± 3 days from day 42 to day 180 and within ±5 days from day 224 to day 270

10.10. Future Research

No additional analysis is planned to be performed on the PK, CTX-1 and Immunogenicity blood samples for possible future research. Any additional research on these samples unspecified by this protocol will require approval from the subjects.

10.11. TOTAL BLOOD LOSS

Total volume of blood drawn for study will not exceed 211.0 mL+ 10 mL per subject for the entire study.

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

For in house blood samples i.e. at 1, 4, 8, and 12 hours on day 1 and of 24 hours on day 2 will be collected through a dead space cannula placed in a forearm vein or direct vein puncture on case to case basis.

Blood sample collection will be followed as per bioanalytical Study Specification Sheet/ lab manual/ equivalent document. Alternatively, if the cannula is blocked or there is difficulty in withdrawing blood through the cannula, blood samples may be withdrawn by a fresh vein puncture using a disposable sterile syringe and a needle at each time of collection.

	Details of Blood Withdrawal		Maximum Amount of blood required
	Blood withdrawn for screening prior to study	:	15.0 mL
+	Blood withdrawn for serum chemistry – albumin-adjusted calcium only on day 1 (pre-dose), 2, 3, 6, 8, 10, 12 and 180	:	20.0 mL
+	Blood volume for PK samples (24 samples of 2.5 mL each)	;	60.0 mL
+	Blood volume for serum CTX-1 samples (16 samples of 3.5 mL each)	:	56.0 mL
+	Blood withdrawn for immunogenicity assessment (10 samples of 5.0 mL each)	:	50.0 mL
+	Blood withdrawn for end of study safety assessment	:	10.0 mL
+	**Total amount of blood required for each subject**	**:**	**211.0 mL + 10 mL (If required)**
Additional blood may also be collected from any subject in the case of inadequate sampling or handling			

10.12. Restrictions

Medications:

Subjects will be instructed to inform the Investigator regarding consumption of any medication (including herbal medicines or vitamin supplements) 28 days prior to dosing on day 01 till completion of the study. If concomitant medication is required during the study, the subjects will be treated accordingly, and a decision to continue or discontinue the subjects will be made by the Investigator, based on (a) the time the medication was administered, (b) pharmacological interaction of concomitant medication with the IP.

Smoking of 10 or more cigarettes per day or equivalent of any other nicotine containing products shall not be allowed for the duration of the study

Subjects will be instructed to abstain from methyl xanthine containing food and beverages, (chocolates, tea, coffee or cola drinks) for at least 72.00 hours (03 days) prior to housing and at least 72.000 hours prior to each follow-up visit .

Subjects will be instructed to abstain from consuming alcohol, grapefruit or its juice and cranberry juice for at least 72.00 hours (03 days) prior to housing and at least 72.000 hours prior to each follow-up visit.

Posture restriction:

Subjects will remain in supine position for at least 04.00 hours post-dose, unless medically necessary due to adverse event or procedurally required. In these cases it will not be considered as protocol deviation. Subjects will be allowed to do normal routine activity avoiding strenuous physical activity from then on.

10.13. Meals

Subjects will be required to fast for at least 10 hours before the start of dosing on day 1. Lunch will be provided after 4.00 hours post dose. Further snacks and dinner will be provided to the subjects at appropriate intervals (i.e. after at 8.00 and 12.00 hours post-dose)

Drinking water will be restricted 1 hour before and 1 hour after dosing. Subjects will be allowed to consume water ad libitum then after.

Note: In case, scheduled meal distribution time, blood sample collection time and vitals coincide, blood sample collection will be performed as per scheduled time.

Non-compliance to above fasting requirements will be recorded as a protocol deviation.

10.14. Subject Compliance Monitoring

The Investigator or his/her designated and qualified representatives, who will administer investigational product only to subjects who are eligible in the study in accordance with the protocol. The investigational product must not be used for reasons other than that described in the protocol. Independent study personnel shall monitor the dosing and perform physical verification of PFS (including droplet formation on inner/outer surface of PFS) immediately after dosing to confirm complete administration of the drug.

If there are any significant irregularities in compliance in the opinion of the Investigator, the subject should be discontinued from the study.

10.15. Precautions to be taken during COVID-19 Pandemic

Subjects will be advised to follow all the precautions during study participation as suggested by site, Investigator and local & international regulatory authorities from time to time till the pandemic continues.
COVID testing will be performed & subject will be managed in line with the clinical site's current COVID prevention of infection management strategy and applicable local government/health guidelines.

11. ASSESSMENTS

11.1. SAFETY ASSESSMENTS

Safety assessment will be carried out as outlined in Section 11 of this protocol and adverse event (AE) or Serious Adverse Event (SAE) information will be collected and reported as mentioned in Section 12 of the protocol.

Adverse event and Serious adverse events as and when occur, will be properly recorded, evaluated, managed and reported from signing informed consent till EOS assessment visit.

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

All subjects will be advised to maintain good oral hygiene, receive dental check-ups as necessary, and immediately report any oral condition such as dental mobility, pain or swelling or non-healing of sores or discharge during study. Any invasive dental procedures required during the study, same will be performed after consultation/ discretion of Investigator.

All AE's will be summarized using appropriate medical coding dictionary.

11.2. LABORATORY ASSESSMENTS

All subjects will have the laboratory samples drawn as outlined in Section 5.2. Laboratory samples for all subjects will be assessed using a either a certified local laboratory or central lab.

Qualified medical staff will review, initial and date all laboratory results. Any laboratory value outside the reference range that is considered clinically significant by the Investigator will be followed as appropriate. Reports from the central laboratory should be filed with the source documents for each subject.

Table No. 1: Clinical Laboratory Tests on Screening

Hematology*	Serum Chemistry	Other blood test	Urine Analysis
Hemoglobin Platelet count RBC WBC count Differential (absolute and percentage) WBC count	ALT AST ALP Total bilirubin Albumin and total proteins BUN[@] (mmol/l), creatinine, electrolytes (sodium, potassium, chloride), glucose, serum calcium, albumin-adjusted calcium, magnesium, and phosphorus	Serology: HIV, HBsAg, HCV	Routine examination: Color, transparency, pH, specific gravity, glucose, ketones, bilirubin, blood, nitrite, urobilinogen leucocyte esterase and protein Microscopic examination: RBC, leucocytes, epithelial cells and bacteria, crystals.) Urine screen for drugs of abuse (Benzodiazepines, Cannabinoids, Amphetamine, Cocaine, Barbiturates, Morphine) Urine screen for Cotinine test
COVID-19 test		Alcohol test (breath analyzer or any suitable test)	

*Additional laboratory parameters may be analyzed as per standard instrument panel.

Table No. 2: Clinical Laboratory Tests on check-in day

Urine screen for drugs of abuse	Urine screen for Cotinine test
Alcohol test (breath analyzer or any suitable test)	COVID-19 test

Table No. 3: Clinical Laboratory Tests on Days 1 (pre-dose), 2, 3, 6, 8, 10, 12, and 180

Albumin-adjusted calcium
Urine screen for cotinine test (only on Days 12 and 180)

Table No. 4: Each follow-up visit

Alcohol test (breath analyzer or any suitable test)

Table No. 5: Clinical Laboratory Tests on Days 28, 42, 63, 90, 119, 147 and 224

Urine screen for cotinine test

Table No. 6: Clinical Laboratory Tests on EOS Assessment

Hematology	Serum Chemistry	Urine Analysis
Hemoglobin Platelet count RBC WBC count, Differential (absolute and percentage) WBC count	ALT AST ALP Total bilirubin Albumin and total proteins BUN[@], creatinine, electrolytes (sodium, potassium, chloride), glucose, serum calcium, albumin-adjusted calcium, magnesium, and phosphorus	Routine examination: Color, transparency, pH, specific gravity, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, leucocyte esterase and protein Microscopic examination: RBC, leucocytes, epithelial cells and bacteria, crystals. Urine screen for cotinine test
Alcohol test (breath analyzer or any suitable test)		

[@]Note: BUN will be reported in the form of Urea in mmol/L.

12. SUBJECT SAFETY

12.1. ASSESSING, RECORDING AND ANALYZING SAFETY PARAMETERS

12.1.1. Adverse Events

As per ICH-GCP E6 (R2) guidelines, adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of IP.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product. All AEs will be followed up to adequate resolution.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" should be captured in the AE section of the (e)CRF pages. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. Subject well-being assessment can be done telephonically if site visits are not possible due to COVID-19 pandemic.

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:

Table: Severity classification of adverse events

Grade	Description
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

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

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.

If AE is on-going at any visit, please follow the subject till the event get resolved or condition gets stabilized.

12.1.2. Serious Adverse Event

As per ICH-GCP E6 (R2), 21CFR 312.32, UK MHRA Guidelines on Good Pharmacovigilance Practices (GVP), serious adverse event (SAE) or serious adverse drug reaction is any medical occurrence that at any dose:

No.	Description
1	Results in death
2	Is life threatening
3	Requires inpatient hospitalization or prolongation of existing hospitalization

4	Results in persistent or significant disability/incapacity
5	Is a congenital anomaly or birth defect
6	Other (Important Medical Events)

Any other important medical event that may not result in death, or is life threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic broncho-spasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be recorded on the appropriate SAE Report form as per the instructions for use, and applicable regulatory requirement.

12.1.3. Abnormal Laboratory Values or Abnormal Clinical Findings

Laboratory abnormalities that are considered clinically significant by the Investigator constitute an adverse event and should be recorded on the Adverse Events eCRF. Sample collection date and time shall be considered as AE onset date and time. Abnormal laboratory values or abnormal clinical findings at the time of screening will be considered as medical history and not AE. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event.

12.2. Assessment of Outcome of Adverse Events

The outcome of the AEs will be assessed and recorded as per the following categories:

- Recovered/resolved;
- Ongoing (Not resolved or stabilized on follow-up);
- Recovered with sequelae;
- Unknown;
- Death.

All AEs will be followed up to adequate resolution.

12.3. Safety Reporting

12.3.1. Adverse Events

All Adverse Events regardless of seriousness or relationship to Investigational Product, spanning from the signature of the informed consent form (i.e., occurring during the screening period even in the absence of any administration of investigational product), up to End of study are to be recorded on the corresponding page of the Case Report Form i.e. AE/ SAE form.

Whenever possible, diagnosis or single syndrome should be reported instead of individual, separate symptoms. The Investigator should specify the date of onset, intensity, action taken with respect to Investigational Product, corrective treatment/therapy given, additional investigations performed, outcome and his/her opinion as to whether there is a reasonable possibility that the Adverse Event was caused by the Investigational Product.

Laboratory or vital sign abnormalities are to be recorded as Adverse Events only if they are medically relevant: symptomatic, requiring corrective treatment, leading to discontinuation and/or fulfilling a seriousness criterion.

12.3.2. Serious Adverse Events

Any AE considered serious by the Investigator or sub-Investigator or which meets the aforementioned criteria is subjected to expedited reporting.

All the SAEs will be reported by the Investigator to the Sponsor / CRO within 24 hours of awareness and followed by written reports within 7 days of first knowledge by the Sponsor, irrespective of causality. In case Investigator fails to report any SAE within the stipulated period, they shall have to furnish the reason for the delay to the satisfaction of the Licensing authority along with the report of the SAE.

Sponsor/ CRO will report fatal or life-threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) to Ethics Committee and applicable regulatory authority as soon as possible, but no later than 7 calendar days of the event. Any additional relevant information must be sent within 8 days of the report.

Sponsor/ CRO will report non-fatal or non-life-threatening SUSARs to Ethics Committee and applicable regulatory authority as soon as possible but no later than 15 days of the event.

Minimum criteria for reporting
Information for final description and evaluation of a case report may not be available within the required time frames for reporting outlined above. Nevertheless, for regulatory purposes, initial reports should be

submitted within the prescribed time as long as the following minimum criteria are met: an identifiable patient; a suspect medicinal product; an identifiable reporting source; and an event or outcome that can be identified as serious and unexpected, and for which, in clinical investigation cases, there is a reasonable suspected causal relationship. Follow-up information should be actively sought and submitted as it becomes available.

12.3.3. SAE Follow-up

Any follow-up information received for the SAE should be analyzed and reported as per the process and timelines mentioned above for initial SAE reporting.

The Investigator should take all appropriate measures to ensure the safety of the subjects, notably they should follow up the outcome of any Adverse Events (clinical signs, laboratory values or other, etc.) until the subject returns to normal or consolidation of the subject's condition;

In case of any Serious Adverse Event, the subject must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized.

This may imply that follow-up will continue after the subject has left the Clinical trial.

12.3.4. Overdose

Overdose is defined as a dose greater than the dose specified in the protocol. Any overdose must be recorded in the AE section of the (e)CRF and the source documents. Any case of overdose leading to SAEs must be reported in an expedited manner using the SAE reporting form. Overdose (with associated symptoms or without any associated symptoms), should be reported as an adverse event using the Preferred Term "OVERDOSE''.

12.4. MEDICAL MONITORING

It is the responsibility of the Investigator to oversee the subject's safety at his site. This safety monitoring will include careful assessment and appropriate reporting of AEs as noted above, as well as the construction and implementation of data and safety-monitoring plan.

13. BIOANALYTICAL PROCEDURES

Denosumab, anti-Denosumab antibody, and neutralizing antibody assessment in human serum will be done at bioanalytical lab using validated analytical methods.

Serum concentrations of denosumab will be measured at central lab by a validated analytical method.

Serum concentrations of CTX-1 will be measured by bioanalytical lab using a validated analytical method.

A separate plan will be prepared for collection, processing and shipment of biological samples. The information on sample handling will be included in Study Specification sheet or as per CRO SOPs [i.e. sample collection, processing and storage requirements (i.e. sample type, collection time, collection tubes, centrifuge duration, speed and temperature requirement, aliquoting, storage temperature, shipments condition etc)].

The procedure for sample handling, storage and analysis for the above samples at lab will be provided in the Study Specification Sheet.

14. STATISTICAL ANALYSIS

All data will be listed and descriptive analysis along with appropriate graphs will be provided by the treatment groups. Categorical data will be summarized using absolute and relative frequencies (counts and percentages) and the summary measures for continuous data will include the number of observations, the arithmetic mean, standard deviation, minimum, median, maximum, CI, geometric mean, and CV as appropriate.

14.1. Sample size justification

It is assumed that the true ratio for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} between ENZ215, EU-sourced Prolia® and US-sourced Prolia® is 0.95 and the between subject coefficient of variation (CV) for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} 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 $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} between ENZ215 and Prolia groups will be within the (80% to 125%) limits. Assuming a drop-out rate of 10% (as this study is a short study in healthy volunteers), at least 207 (69 per group) subjects are required.

14.2. Population for Analyses

PK Population: All subjects who comply sufficiently with the protocol who received the single dose of the study drug and had one pre-dose and at least one post-dose measurement of any of the PK assessment not impacted by any protocol deviations.

PD Population: All subjects who comply sufficiently with the protocol, who received the single dose of the study drug and had one pre-dose and at least one post-dose measurement of any of the PD assessment.

Safety Population: All subjects who received the single dose of the study drug will be included in the safety evaluations.

14.3. Pharmacokinetic Analyses

14.3.1. Serum Pharmacokinetic Parameters

The following primary PK parameters will be calculated for Denosumab in serum, as appropriate: C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}t}$.

The following secondary PK parameters will be calculated for Denosumab in serum, as appropriate: $AUC_{0\text{-}28\ Day}$, T_{max}, T½, and CL/F.

14.3.2. Pharmacokinetic exclusion criteria:

- No value for $AUC_{0\text{-}inf}$, CL/F or $T_{½}$ will 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 will be as below:
  - Three consecutive missing (M) / Non-Reportable (NR) samples in elimination phase may significantly influence the $AUC_{0\text{-}t}$, $AUC_{0\ -\ Day\ 28}$ and elimination phase dependent parameters ($AUC_{0\text{-}inf}$ and $T_{1/2,}$ CL/F). Inclusion of such parameters in the statistical analysis may mislead the final outcome. Hence, $AUC_{0\text{-}t,}$ $AUC_{0\ -\ Day\ 28}$ and elimination phase dependent parameters ($AUC_{0\text{-}inf}$ and $T_{1/2}$) will be excluded.
  - Additionally any subject with at least 3 consecutive Missing (M) / Non-reportable (NR) samples during the absorption phase such subject will be excluded from the pharmacokinetic and statistical analysis. In such a scenario, only plasma concentration versus time data of that subject will 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 will be excluded from the pharmacokinetic and statistical analyses for the assessment of bioequivalence. A subject is considered to have very low serum concentrations if his/her AUC is 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 will be used:

- If the pre-dose concentration is less than or equal to 5% of the corresponding C_{max} value for that subject, the subject's data will be included in all PK measurements and calculations without any adjustment.
- If the pre-dose value is greater than 5% of the corresponding C_{max} value for that subject, the subject's data for the period in question will be excluded from the statistical evaluations (descriptive statistics for that period and ANOVA).

14.3.3. Statistical Analyses of Pharmacokinetic Parameters:

14.3.3.1. Analysis of Variance

Statistical tests like ANOVA, least square means for test and reference formulations, difference between test and reference formulations, inter-subject variability and power will be calculated for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}.

Geometric least square means of test and reference formulations, its ratio, 90% confidence interval for geometric least square mean ratio (ENZ215 / Prolia) and Two One- Sided Tests for 90% confidence interval limits will be calculated for pharmacokinetic parameters.

Bioequivalence between ENZ215 and Prolia will be concluded if the 90% CI of the ratio of the geometric means for AUC_{0-t}, AUC_{0-inf} and C_{max} fall entirely within the (80.00% to 125.00%) limits.

14.3.3.2. Non-Parametric Analysis

A non-parametric analysis will be conducted to test the difference between ENZ215 and Prolia for the PK parameters T_{max} in the original scale.

14.3.4. Serum Pharmacodynamic Parameters

AUEC will be calculated for serum CTX-1.

14.3.5. Analyses of Pharmacodynamic Parameters:

The AUEC will be calculated as the area under the effect curve from baseline until CTX-1 values return to baseline for the first time. An ANCOVA will 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 will be based upon the 95% confidence intervals for the ratio of the geometric means (ENZ215 and Prolia) for AUEC of baseline-corrected serum CTX-1 (i.e.% change from baseline to month 9), which have to be contained entirely within the pre-specified limits of 0.80-1.25.

14.3.6. Safety:

Safety parameters such as incidence of adverse events, clinically significant changes in physical examination findings, safety laboratory analytes (serum chemistry, hematology, and urinalysis), vital signs, and 12-lead electrocardiogram, Anti-Denosumab antibody and Neutralizing antibody assessment will be descriptively summarized, for the treatment groups as appropriate.

Analysis and descriptive methodologies will be further elaborated in the main body of the protocol as well as in the Statistical Analysis Plan for this study.

15. ETHICAL AND REGULATORY STANDARDS

15.1. ETHICAL PRINCIPLES, LAWS AND REGULATIONS

The clinical trial will be conducted as per the principles and requirements of Declaration of Helsinki (Ethical principles for medical research involving human subjects, revised by the 64th WMA General Assembly, Brazil, 2013) and are consistent with the ICH-GCP E6 (R2) guidelines, and applicable regulatory requirements.

15.2. ETHICS COMMITTEE

Sponsor or each participating institution/hospital must provide this protocol (protocol amendment, if applicable) and associated documents for the review and approval to Ethics Committee (EC) registered with regulatory agency, for the formal approval of the study conduct. The decision of the EC, concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of this study. If requested, a progress report during the trial and a summary of the study at the end of the clinical trial will be sent to the EC. The study will not commence until the committee has approved the final version of the protocol.

15.3. INFORMED CONSENT PROCESS

The Investigator or a person designated by the Investigator, and under the Investigator's responsibility, should completely inform the subjects and/or their families describing this study and providing sufficient information to them for making an informed decision about their participation in this study.

All the subjects should be informed to the complete extent possible about the study, in language and terms they are able to understand. The Consent form must be IRB/IEC approved and the subject will be provided the same in local language(s).

Prior to the subject's participation in this study, the written informed consent form must be signed, name filled in and personally dated by the subject and by the person who conducted the informed consent discussion. A copy of the signed and dated written informed consent form will be provided to the subject.

15.4. SUBJECT AND DATA CONFIDENTIALITY

Subject confidentiality along with the information disclosed/provided/produced by the Sponsor during the clinical trial, including, but not limited to, the clinical trial protocol, the (e)CRF, ICFs and results are strictly held in trust by the Sponsor, Sponsor's authorized personnel, Investigator and their staff members. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

However, the submission of this clinical trial protocol and other necessary documentation to the ethics committee is expressly permitted, the EC members having the same obligation of confidentiality. The European Union (EU) General Data Protection Regulation (GDPR) will be followed for data protection and privacy for E.U. submission.

16. DATA HANDLING AND RECORD KEEPING

16.1. CASE REPORT FORMS/ELECTRONIC CASE REPORT FORM

Standard operating procedure are available for all activities performed at CRO relevant to the quality of this study. Designated personnel of CRO will be responsible for implementing and maintaining quality assurance (QA) and quality control system to ensure that the study is conducted, and that data will be documented on the Source Data Location List as per relevant SOPs in compliance with the study protocol and GCP requirements as well as applicable regulatory requirements and local laws, rules and regulations relating to the conduct of the clinical study.

The Clinical Study report will reviewed (QA/QC) as per CRO SOPs.

All clinical data will undergo a 100% quality control check prior to clinical database lock.

Edit checks will then be performed for appropriate database as a validation routine using SAS® or comparable statistical program to check for missing data, data inconsistencies, data ranges, etc. Corrections are made prior to database lock.

16.2. DATA CAPTURE METHODS

CRFs will be produced, stored electronically, and will be made available to the designated study team members. Each CRF will be reviewed and signed by the PI. The final signed CRFs will be provided to the Sponsor in the format as decided upon between CRO and the Sponsor (e.g., compact disc, flashdrive, secure file transfer protocol). This will be documented in the Data Management Plan (if applicable).

All raw data generated in connection with this study, together with the original copy of the final report, will be retained by CROsuntil at least 5 years after the last approval of a marketing application in as ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 5 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the PI/Institution as to when these documents no longer need to be retained.

16.3. DATA MANGEMENT TEAM

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Investigator. During the study, the Investigator must maintain complete and accurate documentation for the study.

The Sponsor will serve as the statistical and data coordinating center for the present study and will be responsible for data management, quality review, analysis, and reporting of the study data.

16.4. RECORD RETENTIONS

It is the responsibility of Sponsor to make arrangements for safe and secure custody of all study related documents and material for a period as defined in the ICH-GCP. These documents should be retained for a longer period of time, if required by an agreement with the Sponsor. In such an instance, it is the responsibility of the Sponsor to inform the Investigator / institution as to when these documents no longer need to be retained.The pharmacokinetic and bioanalytical data will be archived at their respective site.

17. STUDY MONITORING, AUDITING AND INSPECTING

17.1. RESPONSIBILITIES OF INVESTIGATORS

The Investigator(s) and delegated Investigator staff undertake(s) to perform the Clinical Trial in accordance with this Clinical Trial Protocol, guidelines for Good Clinical Practice and the applicable regulatory requirements.

The Investigator is required to ensure compliance with all procedures required by the Clinical Trial Protocol, SOP/in house procedure of CRO and with all study procedures provided by the Sponsor (including security rules).

The Investigator agrees to provide reliable data and all information requested by the Clinical Trial Protocol in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents by Sponsor representatives.

If any circuit includes transfer of data, particular attention should be paid to the confidentiality of the subject's data to be transferred.

The Investigator may appoint such other individuals as he/she may deem appropriate as Sub-Investigators to assist in the conduct of the Clinical Trial in accordance with the Clinical Trial Protocol. All Sub-Investigators shall be appointed and listed in a timely manner. The Sub- Investigators will be supervised by and work under the responsibility of the Investigator. The Investigator will provide them with a copy of the Clinical Trial Protocol and all necessary information.

17.2. RESPONSIBILITIES OF SPONSOR AND STUDY MANAGEMENT

The Sponsor of this Clinical Trial shall take all reasonable steps to ensure proper conduct of the Clinical Trial Protocol as regards ethics, Clinical Trial Protocol compliance, integrity and validity of the data

recorded on the (e)CRFs. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor in maintaining a high level of ethical, scientific, technical and regulatory quality in all aspects of the Clinical Trial.

At regular intervals during the Clinical Trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and subject compliance with Clinical Trial Protocol requirements and any emergent problems. These monitoring visits, will include but not be limited to review of the following aspect: subject informed consent, subject recruitment and follow-up, Source data verification, SAE documentation and reporting, AEs with pre-specified monitoring documentation and reporting, AE documentation, IP allocation, subject compliance with the IP regimen, IP accountability, concomitant therapy use and quality of data.

17.3. AUDITING AND INSPECTING

The Investigator will permit study-related monitoring, audits and inspections by the EC, the Sponsor, government regulatory bodies and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The Investigator agrees to allow the auditors or inspectors to have direct access to subject's study records for review, being understood that these personnel are bound by professional secrecy, and as such will not disclose any personal identity or personal medical information. The confidentiality of the data verified and the protection of the subjects should be respected during these inspections.

The Investigator will ensure the capacity for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, study documents etc.). Participation as an Investigator in this study implies acceptance of potential inspection by government regulatory authorities and quality assurance offices. As soon as the Investigator is notified of a future inspection by the authorities, he/she will inform the Sponsor and authorize the Sponsor to participate in these inspections. Any result and information arising from the inspections by the regulatory authorities will be immediately communicated by the Investigator to the Sponsor.

17.4. QUALITY CONTROL AND QUALITY ASSURANCE

The Investigator by careful planning, assigning responsibilities to well qualified study personnel, through continuous review, verifies and maintains desired level of quality in the study process.

Sponsor or the Sponsor's designee will monitor the study and site activity while ongoing, to verify that the:

- Data are authentic, accurate and complete
- Safety and rights of the subjects are being protected

- Study is conducted in accordance with the currently approved protocol and amendments and any other study agreements, GCP, and all applicable regulatory requirements

The pharmacokinetic and bioanalytical site will be responsible for quality assurance audit of pharmacokinetic and bioanalytical data respectively.

18. ADMINISTRATIVE PROCEDURES

The Investigator must maintain confidentiality of all study related documents, and takes measures to prevent accidental or premature destruction of these documents. The retention of the study related documents is depicted in Section 16.4 (RECORD RETENTIONS) of the present protocol.

18.1. PROTOCOL AMENDMENTS

Any change or addition to the protocol, other than administrative ones (i.e. typographical or logistical), can only be made in a written protocol amendment that must be approved by Sponsor, applicable regulatory where required, and the EC. Only amendments that are required for subject safety may be implemented prior to EC approval.

Notwithstanding the need for approval of formal protocol amendments, the Investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a violation of the protocol. In such cases, Sponsor should be notified of this action and the EC should be informed immediately.

Changes to the protocol affecting only administrative aspects of the study do not require formal protocol amendments or EC approval but the EC must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and EC approval that can be treated as administrative amendments include:

- Changes in the staff used to monitor trials
- Changes in shipping address for (e)CRF

18.2. PROTOCOL DEVIATIONS AND VIOLATIONS

According to FDA Inspectional Manual, the term “Protocol Deviation” is “A protocol deviation/violation that is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change”. A deviation or violation is any noncompliance with the requirements of clinical trial protocol, SOPs, ICH-GCP-E6 (R2) guidelines. The noncompliance may be either on the part of the

subject, the Investigator, or the study site staff. As a result of deviations or violation, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to observe vigilance to identify and report deviations immediately upon identification of the protocol deviation. All deviations must be promptly reported to Sponsor through written communication. All deviations from the protocol must be addressed in study subject source documents. A completed copy of the protocol deviation form must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local EC, if required, per their guidelines. The site Investigator / study staff is responsible for adhering to EC requirements.

18.3. INSURANCE COMPENSATION

The Sponsor certifies that it has taken a liability insurance policy covering this clinical trial. The insurance policy is in accordance with local laws and requirements. A copy of the insurance certificate will be provided to the EC. In the case of an injury occurring to the subject during the study, free medical management will be provided to the subjects as long as required or till such time it is established that the injury is not related to the clinical study, whichever is earlier.

18.4. INDEMNITY AGREEMENT AND STUDY FINANCES

Sponsor undertakes to maintain an appropriate clinical study insurance and indemnity policy.

Deviation from the study Protocol-Especially the prescription of a dose other than that scheduled in the study Protocol, other modes of administration, other indication, and longer treatment periods – are not permitted and shall not be covered by the statutory subject Insurance scheme.

18.5. PREMATURE DISCONTINUATION OF THE STUDY

Sponsor on the basis of new information regarding safety or efficacy, reserves the right to terminate the clinical study at any time. Reasons for termination include, but not limited to, following:

a) Unacceptable safety issues
b) Additionally,
   a. Investigator can terminate the study for the safety of subjects.
   b. EC can terminate the study for the safety of subjects and major violations of ethical considerations.
   c. Subsequent review of serious, unexpected, and related AE's by the medical monitor, EC, the Sponsor(s), or regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site. The study Sponsor(s) retain the authority to

suspend additional enrolment and study interventions/administration of study product for the entire study, as applicable.

c) If the Investigator has received from the Sponsor all investigational products, means and information necessary to perform the clinical trial and has not included any subject after a reasonable period of time mutually agreed upon, site can be prematurely closed.

If the study is terminated, all the measures will be taken to ensure that a follow-up visit is preformed to complete end of study assessment visit.

18.6. PROPERTY RIGHTS AND DATA PROTECTION

All information, documents and investigational product provided by the Sponsor or its assignee are and remain the sole property of the Sponsor. The Investigator shall not mention any information or the product name in any application for a patent or for any other intellectual property rights. All the results, documents and inventions, which arise directly or indirectly from the clinical trial in any form, shall be the exclusive property of the Sponsor. Any Investigator involved with this study is obliged to provide the Sponsor with complete test results and all data derived from the study. The Sponsor may use or exploit all the results at its own discretion, without any limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the clinical trial.

The data collected during the entire trial will be included in the Sponsor database and shall be treated in compliance with the SOPs prepared according to applicable laws and regulations. When archiving or processing the data, Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.

18.7. Direct Access to Source Data/Documents

Investigator/CRO will ensure that the Sponsor, Research Ethics Committee and inspection by domestic and foreign regulatory authorities will have direct access to all study-related sites, source data/documents, and reports for the purpose of monitoring and auditing (ICH[E6][R2]). In the event that other study-related monitoring should be done by other parties, they will be required to sign a confidentiality agreement prior to any monitoring and auditing.

18.8. CLINICAL STUDY REPORT

A final clinical study report will be prepared according to the ICH E3 guideline on structure and content of Clinical Study Report. A Final Clinical Study Report will be prepared regardless of whether the study is completed or prematurely terminated. The final report will be prepared according to the eCTD

(electronic Common Technical Document) format. The deviations from the protocol will be documented as per CRO SOPs/ study specific plan/ equivalent document and presented in the final report.

18.9. PUBLICATION POLICY

It is the responsibility of the Sponsor to register this trial applicable registry. On the basis of the statistical and clinical evaluation of the pooled results across the study centers, a clinical study report will be prepared. This can form the basis of a manuscript for publication in a peer-reviewed journal. The Sponsor will hold the right to publish the results of present study at any time. An Investigator may seek permission to publish results of the study from the Sponsor.

19. REFERENCES

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2. USFDA Prescribing Information of Prolia® (denosumab) Injection, for subcutaneous use; Amgen Inc. One Amgen Center Drive Thousand Oaks, California 91320-1799, May/2021
3. The use of the WHO-UMC system for standardized case causality assessment
4. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Guidelines: Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2).
5. Declaration of Helsinki (Ethical principles for medical research involving human subjects, revised by the 64th WMA General Assembly, Brazil, October 2013).
6. General Data Protection Regulation (GDPR), European Union.
7. Common Terminology Criteria for Adverse Events [CTCAE]; National Cancer Institute, U.S. Department of Health and Human services, Version 5.0, Published: November 27, 2017.
8. USFDA's Guidance for Industry: Statistical Approaches to Establishing Bioequivalence; January 2001.
9. Guideline on the Investigation of Bioequivalence Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr ** effective dated 1 August 2010.
10. EMA Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) pandemic, version 04 (04 Feb 2021).
11. FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency Guidance for Industry, Investigators, and Institutional Review Boards, *updated on August, 2021*.
12. United States Food and Drug Administration. Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. April 2015. https://www.fda.gov/regulatory-information/search-fda-guidancedocuments/

20. APPENDICES

SUBJECT INFORMATION SHEET AND INFORMED CONSENT FORM