Statistical Analysis Plan (SAP)

Trial Full Title	*SENolytics to Improve Osteoporosis therapy: a Randomised controlled clinical trial (The SENIOR Trial)*
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1 SAP Signatures

I confirm my approval of the Statistical Analysis Plan (SAP) titled *"SENolytics to Improve Osteoporosis Therapy: The SENIOR Trial"*, version 1.6 dated 01.05.2026.

Independent Statistician

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3 Abbreviations and Definitions

AE	Adverse Event
ANCOVA	Analysis of Covariance
BAP	Bone-specific alkaline phosphatase
BMD	Bone Mineral Density
CTX	C-terminal telopeptide of type I collagen
D+Q	Dasatinib + Quercetin
DXA	Dual-energy X-ray Absorptiometry
HR-pQCT	High Resolution Peripheral Quantitative Computed Tomography
IMP	Investigational Medical Product
ITT	Intention To Treat
LOC	Level of Confidence
LOCF	Last Observation Carried Forward
MAR	Missing At Random
MMRM	Mixed Model for Repeated Measures
NR	Nicotinamide Riboside
P1NP	Procollagen type 1 N-terminal propeptide
PP	Per-Protocol
REML	Restricted Maximum Likelihood
SAP	Statistical Analysis Plan
SASP	Senescence-associated secretory phenotype
TRAcP	Tartrate-Resistant Acid Phosphatase
vBMD	Volumetric Bone Mineral Density

4 Introduction

4.1 Preface

Osteoporosis and osteopenia are common age-related conditions that are partly caused by the accumulation of senescent cells and changes in cellular NAD^+ metabolism. Preclinical studies have shown that senolytics (dasatinib and quercetin) and NAD^+ precursors (nicotinamide riboside) can reduce the burden of senescent cells, restore the function of osteoblast lineage, and slow down age-related bone loss. This trial aims to investigate whether 20 weeks of treatment with dasatinib plus quercetin or nicotinamide riboside can improve bone remodelling in older adults with low bone mass.

4.2 Scope of the analyses

These analyses will assess the effects of intermittent senolytic therapy with dasatinib (D) plus quercetin (Q) and daily nicotinamide riboside (NR) to reduce bone resorption, as assessed by the percentage change in circulating CTX from baseline (week 1) to week 21. In addition, these analyses will investigate the effect of the interventions on bone formation as reflected by the bone formation marker P1NP, which serves as the sole secondary biochemical endpoint at this stage.

Further exploratory analyses will include additional bone turnover markers—such as osteocalcin, BAP, and tartrate-resistant acid phosphatase—recognising that these will not be analysed initially but may be incorporated in future statistical evaluations based on feasibility and available funding. Exploratory endpoints will also assess changes in bone mineral density using dual-energy X-ray absorptiometry (DXA), alterations in bone microarchitecture and geometry using high-resolution peripheral quantitative computed tomography (HR-pQCT), and systemic markers of cellular senescence, including senescence-associated secretory phenotype (SASP) factors. Additional exploratory outcomes include measures of senescent cell burden in peripheral blood and bone tissue, as well as assessments of muscle function and whole-body composition.

Together, these analyses aim to determine whether interventions that target cellular senescence or promote NAD^+ metabolism can improve skeletal health and reduce markers of biological aging in older individuals with low bone mass.

The trial protocol (v3.0, 10-03-2025) specified an ANCOVA model as the primary analysis method. Following review by the independent statistician prior to transfer of data to the analysis file, the primary estimator has been updated to a Mixed Model for Repeated Measures (MMRM), in

accordance with the EMA Guideline on Missing Data in Confirmatory Clinical Trials (EMA/CPMP/EWP/1776/99 Rev. 1, 2010), which recognises the statistical analysis plan as the appropriate vehicle for pre-specifying analysis methods, and ICH E9(R1) (2019), which distinguishes between the estimand (unchanged: percentage change in CTX from baseline to week 21) and the estimator (updated: MMRM). This SAP has been finalised and approved by the independent statistician prior to initiation of any data analyses. This update does not alter the primary estimand, endpoint definition, analysis population, or significance level, and is expected to provide equal or greater statistical efficiency than the originally specified ANCOVA under the missing-at-random assumption. The ANCOVA model specified in the protocol is retained as a pre-specified sensitivity analysis.

5 Study Objectives and Endpoints

5.1 Study Objectives

The objective of this study is to determine whether intermittent senolytic therapy with dasatinib plus quercetin (D+Q) or daily nicotinamide riboside (NR) supplementation influences bone resorption and/or bone formation in older women and men with low bone mass and increased fracture risk.

5.2 Endpoints

5.2.1 Primary endpoint

Percentage change in plasma CTX from baseline to week 21.

5.2.2 Secondary endpoints

Percentage change in plasma P1NP from baseline to week 21.

5.2.3 Exploratory endpoints

Exploratory analyses may be conducted to further characterize clinical, biological, or patient reported outcomes collected during the study. These analyses are hypothesis-generating and will be interpreted without confirmatory intent.

Exploratory imaging endpoints

- Change in bone mineral density assessed by DXA scanning
- Changes in tibial (leg) and radial (forearm) bone geometry and bone microarchitecture in

trabecular and cortical bone, including volumetric bone mineral density (vBMD), trabecular thickness (Tb.Th), trabecular bone volume per tissue volume (Tb BV/TV), cortical thickness (Ct.Th) and cortical porosity (Ct.Po) assessed by high resolution peripheral quantitative computed tomography (HR-pQCT scan)

- Estimated bone strength ("Failure load") by finite element analysis (HR-pQCT scan)

Exploratory senescence and molecular endpoints

- Circulating SASP factors
- Peripheral blood p16INK4a expression
- Senescent cell burden in bone biopsy samples

Exploratory functional endpoints

- Grip strength
- 30-second Sit-To-Stand test
- Timed-Up-and-Go test
- Lean mass and fat mass from DXA
- Change in body mass index/weight (including data from weeks 5, 13 and 21)

Exploratory biochemical endpoints

- Percentage change in osteocalcin
- Percentage change in bone-specific alkaline phosphatase
- Percentage change in tartrate-resistant acid phosphatase

Exploratory biochemical markers will be assessed at weeks 5, 13, and 21. The primary and secondary endpoint will additionally be assessed at weeks 5 and 13 to evaluate early changes in bone resorption.

6 Study Methods

6.1 General Study Design and Plan

This study is a 20-week, randomised, open-label, parallel-group clinical trial evaluating whether intermittent senolytic therapy with D+Q or daily NR supplementation influences bone metabolism in older adults with low bone mass. Participants are randomised in a 1:1:1 ratio to receive D+Q, NR, or no treatment.

6.2 Inclusion-Exclusion Criteria and General Study Population

6.2.1 Inclusion criteria

- Men and women (menopause > 5 years and FSH and LH in the postmenopausal range) aged 60-90 years with increased fracture risk according to WHO 10 years absolute Fracture Risk Assessment Tool (FRAX).
  - Osteopenia (ICD10 DM858A) based on a T-score ≤ -1 and >-2.5 at the total hip/femoral neck, or lumbar spine, with or without a fragility fracture at any time (excluding hip and vertebral fractures within the last 2 years) (FRAX ranging from 7-70)
  - osteoporosis (ICD10 DM819) based on a T-score >-3 and ≤ -2.5, which includes candidates suitable for conventional osteoporosis therapies, but who prefer to participate in the trial, despite being candidates for conventional osteoporosis therapy, or candidates who cannot be treated with conventional therapies due to contraindications.
- Ability to provide informed consent

6.2.2 Exclusion criteria

Technical or Sampling Limitations

- Inability to perform DXA of hip/spine (e.g., due to prosthesis)
- Unable to provide fasting blood samples
- Unable to take oral medication
- BMI ≥ 40

Medical Conditions

- Primary hyperparathyroidism
- Vitamin D deficiency (<50 nM; re-test allowed after correction)
- Disorders affecting bone metabolism (e.g., uncontrolled thyrotoxicosis, CKD with eGFR <30, liver dysfunction, rheumatism, celiac disease, malabsorption, hypogonadism, severe COPD, hypopituitarism, Cushing's, uncontrolled diabetes with HbA1c >58 mmol/mol)
- QTc >470 ms.
- Clinically significant abnormal blood counts
- Heart failure, malignancy, or other conditions deemed high-risk by the investigator

Medication Restrictions

- Use of antiresorptive or bone anabolic drugs in the past 2 years (5 years for zoledronic acid)
- Current use of medications affecting bone metabolism (e.g., systemic glucocorticoids, anabolic steroids)

- Inability to pause interacting drugs during D+Q dosing (e.g., digoxin, lithium, statins, methotrexate, warfarin, etc.)
- Use of drugs with narrow therapeutic windows metabolised by CYP3A4, CYP2C8, CYP2C9, or CYP2D6
- Use of proton pump inhibitors, unwilling to pause for 2 days before/during dosing
- Anti-arrhythmic drugs known to prolong QTc
- Tyrosine kinase inhibitors
- Antiplatelet agents that cannot be paused during dosing/biopsy periods
- Antimicrobials with known interactions (e.g., azoles, macrolides, antivirals)

Other

- Known allergy to dasatinib, quercetin, or nicotinamide riboside
- Any condition that may interfere with study participation or safety

Eligibility will be assessed by a delegated investigator who is a trained physician.

6.3 Randomisation and Blinding

Randomisation of the participant will occur immediately after all baseline assessments are completed and eligibility is confirmed at the baseline visit. Randomisation is performed using the REDCap® randomisation module and follows a fixed 1:1:1 allocation ratio to the three treatment groups (D+Q, NR, Control). Block randomisation with block sizes of 3 and 6 is used to ensure that the 1:1:1 ratio is consistently maintained throughout enrolment. The allocation is stratified by age category (below or above 75 years) and sex, thereby promoting comparability across clinically relevant subgroups. Because this is an open-label trial, both participants and investigators know the treatment assignments. Blinding is limited to the independent statistician, who will receive data with anonymised group labels such as Group A, B, or C. The statistician will not know which label corresponds to which treatment while conducting the analyses. All three pairwise group comparisons will be calculated and reported during the blinded phase. The classification of comparisons as primary or exploratory will be finalised only after the group labels are revealed to the statistician at the end of the blinded analyses.

6.4 Study Assessments

Study assessments are conducted at baseline and at approximately week 5, week 13, and week 21, according to pre-specified windows defined in the protocol.

Table 1. Study overview

	Day 0 Screening	Day 1 Week 1 Baseline	Day 29 Week 5	Day 57 Week 13	Day 141 Week 21 End visit
Informed Consent	X				
Assessment of Inclusion and exclusion criteria	X				
Randomization		X			
Blood samples					
Screening and control	X		X	X	X
Bone turnover markers		X	X	X	X
Scans					
DXA scan	X	(X^1)			X
HR-pQCT scan		X			X
Other investigations					
ECG	X	(X^2)	(X^2)	(X^2)	X
Weight and height	X	X	X	X	X
Bone biopsy					X

(X^1) DXA repeated at baseline only if screening scan was performed >4 weeks prior to randomisation.
(X^2) ECG performed only if clinically indicated (symptoms or deviations) or if randomised to D+Q.

Table 2. Time Windows

Visit (target day)	Lower bound (days)	Upper bound (days)
Screening (0)	N/A	N/A
Baseline (Day 1)	N/A	120 days after screening
Month 1 (29)	24	34
Month 3 (57)	52	62
Endvisit, Month 5 (141)	136	146

6.4.1 Biochemical Assessments

At all scheduled timepoints (baseline, week 5, week 13, week 21), fasting serum and plasma samples will be collected for:

- **Primary endpoint:** CTX
- **Secondary endpoint:** P1NP
- **Exploratory bone turnover markers (not analysed initially):**
    - Osteocalcin, Bone-specific alkaline phosphatase (BAP), Tartrate-resistant acid phosphatase (TRAcP)

All exploratory markers will be stored under validated biobanking conditions to allow future analysis.

6.4.2 Imaging Assessments (Exploratory)

Imaging assessments are performed at baseline and week 21:

- **DXA scans** (lumbar spine, total hip, femoral neck, and whole-body composition)
- **HR-pQCT scans** of distal radius and tibia
- **Finite element analysis** for estimated bone strength (derived from HR-pQCT)

These outcomes support an exploratory investigation of structural skeletal changes.

6.4.3 Senescence and Molecular Assessments (Exploratory)

Assessed at baseline and week 21 when data are available:

- Peripheral blood p16INK4a expression (senescent T-cell biomarker)
- Serum SASP factors
- NAD(H) and related metabolic markers
- Bone biopsy from the iliac crest for senescent cell quantification (subset)

These endpoints enable mechanistic exploration but are not included in initial efficacy analyses.

6.4.4 Physical Function Assessments and Body Composition (Exploratory)

Assessed at baseline and week 21 when data are available:

- Hand grip strength
- 30-second Sit-To-Stand test
- Timed-Up-and-Go test
- Body weight: Measured as body mass index (kg/m^2).
- Body composition: Measured using Whole body DXA-scans and include Weight/BMI ($kg/kg/m^2$), Total body mass fat (g), Total body lean mass (g), Abdominal subcutaneous fat (g) (Android fat mass – visceral fat mass)

7 Sample Size

The sample size was based on the primary endpoint: percentage change in plasma CTX from baseline to week 21. At the time of study planning, no clinical trials had evaluated the effects of dasatinib plus quercetin or nicotinamide riboside versus control on human bone turnover. The power calculation therefore used data from a comparable 20-week randomised trial investigating the effects of beta-blocker therapy on bone turnover markers in postmenopausal women.

Based on these assumptions, detecting an approximately 18.3% difference in CTX between either intervention group and the control group using an ANCOVA model adjusting for baseline CTX would require 40 participants per group. This provides 90% power at a two-sided significance level of 0.025, which is appropriate for a three-group comparison. A total of 120 participants was therefore determined to be sufficient for the study's primary objective.

This sample size allows for an anticipated withdrawal rate of approximately 10% over the 20- week study period. The calculation applies only to the primary endpoint and does not account for exploratory endpoints, secondary analyses, or additional repeated measurements obtained at weeks 5 and 13.

Although the original power calculation was based on an ANCOVA model comparing baseline to week 21 values, the primary efficacy analysis will be conducted using a mixed model for repeated measures (MMRM) including all post-baseline measurements. The MMRM approach utilises the longitudinal structure of the data and is expected to provide comparable or improved statistical efficiency relative to a single time-point ANCOVA analysis.

8 General Analysis Considerations

8.1 Timing of Analyses

The final analysis will be performed when all participants have completed the week 21 visit or have withdrawn from the study. No interim analyses will be undertaken. Plasma CTX and P1NP collected at weeks 5 and 13 will not be evaluated prior to study completion and will only be included in the final dataset.

All study data will be transferred to the analysis file after the last patient completes the final visit and after this Statistical Analysis Plan has been finalised and approved.

8.2 Analysis Populations

Two primary analysis populations will be defined for this study: the Intention-to-Treat (ITT) Population and the Safety Population. In addition, a Per-Protocol (PP) Population will be defined for

exploratory sensitivity analyses. All analyses described in this Statistical Analysis Plan will be performed on the appropriate population as specified below.

8.2.1 Intention-to-Treat (ITT) Population

The ITT Population will comprise all participants who have been randomised, irrespective of treatment adherence, completion status, or protocol deviations. Participants will be analysed according to the treatment group to which they were originally randomised.

The ITT Population will serve as the primary population for the evaluation of all efficacy endpoints, including the primary endpoint (percentage change in CTX from baseline to week 21) and the secondary endpoint (percentage change in P1NP from baseline to week 21). Measurements collected at week 5 and week 13 will be included in the final dataset but will not be used for decision-making during the study conduct.

8.2.2 Safety Population

The Safety Population will include all participants who have received at least one dose of investigational treatment (D+Q or NR). Participants in the control group (no treatment) will be included in the Safety Population only for assessment of adverse events and clinical safety data collected during scheduled study visits.

Safety analyses will be conducted according to the treatment received.

8.2.3 Per-Protocol (PP) Population

The PP Population is a subset of the ITT Population and comprises all randomised participants who completed the final trial visit (Week 21) and achieved at least 80% treatment compliance with their allocated intervention (D+Q or NR). This threshold ensures meaningful biological exposure while retaining sufficient participants for exploratory analyses.

The PP Population will be used for exploratory sensitivity analyses of the primary endpoint (percentage change in CTX) and the secondary endpoint (percentage change in P1NP) to assess the robustness of the observed biological effects. These analyses are supportive and will not replace the confirmatory ITT analysis.

Participants will be excluded from the PP Population if they have major protocol deviations likely to compromise the biological interpretability of bone turnover markers. Such deviations will be assessed using pre-specified, objective criteria and applied consistently across treatment groups. Major deviations include:

- **Initiation of prohibited bone-active medication** after randomisation (e.g., bisphosphonates, denosumab, anabolic osteoporosis therapy, or systemic glucocorticoids >5 mg/day for >2 weeks).
- **Any fracture during the study period**, due to its known impact on bone turnover markers.
- **Non-adherence to the required overnight fast (>8 hours)** prior to any CTX/P1NP measurement.
- **Major intercurrent events** expected to influence bone turnover (e.g., major surgery or prolonged immobilisation >1 week).

Additional deviations judged to substantially compromise the interpretability of CTX or P1NP may also lead to exclusion.

PP analyses will use the same MMRM model and covariates as the ITT analysis.

8.3 Covariates and Subgroups

Covariates and subgroup factors have been prespecified to explore potential sources of variability and to improve the precision of treatment effect estimates. These factors will be incorporated into statistical models as outlined for each analysis type.

8.3.1 Covariates

For all primary and secondary efficacy analyses, the following covariates will be included:

- Baseline value of the relevant endpoint (baseline CTX for the primary analysis; baseline P1NP for the secondary analysis)
- Age (continuous)
- Sex (male/female)

These covariates are selected based on their known associations with bone metabolism and to improve model efficiency by accounting for between-participant variability.

8.3.2 Subgroups

Exploratory subgroup analyses may be conducted to evaluate potential heterogeneity of treatment effects. For each subgroup, both the scientific rationale and operational definition are prespecified as follows:

Senescent cell burden (high vs low p16INK4a)

Rationale: Senescence burden may modify the skeletal response to senolytics, as suggested in prior human studies.

Definition: Participants will be divided into tertiles based on baseline peripheral blood p16INK4a expression. For subgroup analyses, participants in the highest tertile will be compared with those in the lower two tertiles.

Baseline BMD category (osteopenia vs osteoporosis)

Rationale: Bone density status may influence skeletal responsiveness to senolytics or NR.

Definition: Categorised as osteopenia (T-score ≤ -1 and >-2.5) vs osteoporosis (T-score ≤ −2.5), based on DXA.

Renal function (eGFR strata)

Rationale: Renal function impacts bone turnover marker clearance.

Definition: eGFR ≥ 60 vs 30–59 ml/min/1.73 m².

Age group (<75 / ≥75 years)

Rationale: Age modifies both senescence burden and bone turnover dynamics.

Definition: <75 years versus ≥75 years.

Sex

Rationale: Bone turnover and drug metabolism differ between men and women.

Definition: Male versus female.

BMI category

Rationale: Body composition affects bone turnover and D+Q pharmacokinetics.

Definition: BMI <25 (normal), 25–29.9 (overweight), ≥30 (obese).

Smoking status

Rationale: Smoking adversely affects bone health and turnover.

Definition: Current, former and never smoker.

Cumulative exposure (exploratory): Pack-years will additionally be evaluated as a continuous variable and categorised as low vs high exposure using a median split.

Physical activity level

Rationale: Physical activity influences bone turnover and musculoskeletal loading, which may modify skeletal responses to senolytics or NR. Differences in habitual and pulse-raising activity could therefore contribute to variability in treatment effect.

Definition: Participants will be categorised using self-reported habitual activity:

Daily activity level categorised as:

- **Low:** <30 minutes/day
- **Moderate:** ~30 minutes/day
- **High:** >30 minutes/day

Pulse-raising activity (exploratory): Weekly frequency of pulse-raising activity categorised as:

- **None (0/week)**
- **Low (1–2/week)**
- **Moderate (3–4/week)**
- **High (5–7/week)**

  This variable will additionally be evaluated as a continuous exposure (0–7 times/week).

Where conducted, subgroup analyses will be performed by including a treatment-by-subgroup interaction term in the relevant model. These analyses are exploratory and will be interpreted descriptively without adjustment for multiplicity.

8.4 Missing Data

For the primary analysis based on a mixed model for repeated measures (MMRM), missing data will be handled implicitly through likelihood-based estimation under the assumption that data are missing at random (MAR). All available post-baseline CTX measurements will contribute to the model estimation without the need for explicit imputation of missing values.

A sensitivity analysis based on an ANCOVA model comparing percentage change in CTX from baseline to week 21 will additionally be performed. In this analysis, missing week 21 CTX values will be imputed using the last available post-baseline CTX measurement (last observation carried forward, LOCF).

For the secondary endpoint (percentage change in P1NP from baseline to week 21), missing values will be handled using the same MMRM framework as applied for the primary endpoint. All available post-baseline measurements will be included in the model without explicit imputation.

Participants with no post-baseline measurements for the relevant endpoint will be excluded from the analysis of that endpoint.

No imputation will be performed for exploratory endpoints, including biochemical markers other than CTX and P1NP, imaging outcomes, senescence-related markers, and functional assessments. Missing data for these exploratory outcomes will be summarised descriptively. Sensitivity analyses may be undertaken if patterns of missingness raise concerns about potential bias.

Patterns of missingness will be explored descriptively to assess the plausibility of the missing-at-random assumption.

8.5 Multiple Testing

The study is designed to allow confirmatory inference for the primary endpoint only. The primary analysis of the percentage change in CTX from baseline to week 21 will be conducted at a two-sided significance level of 2.5%, as specified in the sample size calculation. No additional adjustment for multiplicity will be applied to the primary endpoint.

The secondary endpoint (percentage change in P1NP from baseline to week 21) and all exploratory endpoints will be analysed descriptively or using nominal two-sided 5% significance levels without adjustment for multiple comparisons. These analyses are considered exploratory and hypothesis-generating, and results will be interpreted with appropriate caution. No multiplicity adjustments will be applied to subgroup analyses or sensitivity analyses.

9 Summary of Study Data

All continuous variables will be summarised using the following descriptive statistics by randomised group: number of non-missing observations (n), median, and interquartile range. Categorical variables will be summarised by frequency and percentage using the number of non-missing observations as the denominator. Summary tables will be constructed with a column for each treatment group in the order (D+Q, NR, Control) and will be annotated with the relevant population size for each table, including the number of missing observations where appropriate.

9.1 Subject Disposition

Subject disposition will be summarised using counts and percentages for the following categories:

- Screening failures
- Randomised participants
- Participants completing the week 21 visit
- Participants with early withdrawal (including reason for withdrawal)
- Participants included in the ITT Population
- Participants included in the Safety Population

A CONSORT-style flow diagram will be produced to illustrate the disposition of subjects from screening through to study completion.

9.2 Derived variables

Derived variables for efficacy and exploratory analyses will include:

Bone turnover markers

- Percentage change in CTX from baseline to week 5, week 13, and week 21
- Percentage change in P1NP from baseline to week 5, week 13, and week 21

Percentage change from baseline is calculated as: ((post-baseline value − baseline value) / baseline value) × 100.

HR-pQCT-derived variables

- Total volumetric bone mineral density (Tot.vBMD)
- Cortical thickness (Ct.Th)
- Cortical porosity (Ct.Po)
- Trabecular bone volume fraction (Tb.BV/TV)
- Trabecular thickness (Tb.Th)
- Finite element analysis-derived failure load

DXA-derived variables

- Lumbar spine BMD
- Total hip BMD
- Whole-body fat mass
- Whole-body lean mass
- BMI (derived from measured height and DXA/clinical weight)

Percentage or absolute changes will be calculated as appropriate using standard formulas and reported according to the conventions specified in Section 11.

9.3 Demographic and Baseline Variables

Demographic and baseline characteristics will be summarised for each treatment group. Variables will include:

- Sex
- Age
- Height and weight (including BMI)
- Baseline CTX and P1NP
- Baseline BMD at lumbar spine, total hip, and femoral neck
- Baseline HR-pQCT measures (trabecular and cortical indices)
- Baseline physical activity level
- Smoking status
- Renal function (eGFR)
- Relevant medical history
- Prior fracture history (yes/no)

Descriptive summaries will present the number of non-missing observations, mean and standard deviation, median and interquartile range for continuous variables, and frequencies and percentages for categorical variables.

9.4 Concurrent Illnesses and Medical Conditions

Concurrent medical conditions recorded at baseline will be summarised using system organ class and preferred terms. Conditions will be coded using standard classifications (e.g., ICD-10 where applicable). Frequencies and percentages will be presented by treatment group.

9.5 Treatment Compliance

Treatment compliance will be summarised for participants allocated to the D+Q and NR treatment groups. Compliance will be assessed using the number of investigational tablets dispensed and returned at each visit, supplemented by information recorded in the participant diary. Compliance assessments will additionally incorporate information obtained during contacts between scheduled visits, including any reports of missed doses or deviations from the prescribed regimen.
For participants in the control group (no treatment), adherence to scheduled study visits and completion of protocol-required assessments will be summarised descriptively.

10 Efficacy Analyses

Primary and secondary efficacy analyses are performed on an intention-to-treat basis as detailed below. Exploratory per-protocol analyses will also be performed using the PP Population defined in Section 8.2.3.

10.1 Primary Efficacy Analysis

Primary mixed model for repeated measures (MMRM)

The primary endpoint is the percentage change in plasma CTX from baseline (week 1) to week 21. This will be analysed using an MMRM on log-transformed CTX values, including all available post-baseline measurements (weeks 5, 13, and 21).
Model specifications:

- Outcome: log-transformed CTX
- Fixed effects: treatment group (D+Q, NR, Control), time (categorical), treatment × time interaction
- Covariates: baseline CTX, age, sex

- Covariance structure: unstructured marginal covariance matrix at participant level
- Estimation: restricted maximum likelihood (REML)
- Inference: Kenward–Roger approximation for confidence intervals and degrees of freedom

If the model fails to converge, alternative covariance structures (e.g., autoregressive or compound symmetry) or a simplified random-intercept model may be used. Model assumptions will be evaluated using QQ-plots, residual-versus-fitted plots, and, where relevant, random-effect BLUPs. If assumptions are violated, alternative transformations or analyses on the raw scale may be considered.

Results will be presented as estimated marginal means with standard errors, 95% confidence intervals, and nominal p-values. As the model operates on the log scale, estimated treatment contrasts will be back-transformed to yield geometric mean ratios and the corresponding percentage differences, presented with 95% confidence intervals. To ensure that analyses remain blinded, we will compute and report all three pairwise comparisons: D+Q versus Control, NR versus Control, and D+Q versus NR. We define D+Q versus Control and NR versus Control as the primary comparisons, while D+Q versus NR will be considered exploratory. We will apply this classification once the treatment group labels are revealed to the statistician after the blinded analyses are complete.

Sensitivity ANCOVA analysis

A sensitivity analysis will be performed using an ANCOVA model, with the percentage change in plasma CTX from baseline (week 1) to week 21 as the outcome.

Model specification:

- **Outcome:** percentage change in CTX from baseline to week 21
- **Fixed effect:** treatment group (D+Q, NR, Control)
- **Covariates:** baseline CTX, age, sex

Missing week 21 CTX values will be imputed using the last post-baseline CTX measurement for participants with at least one post-baseline value, in accordance with the protocol.

Model assumptions will be assessed using QQ-plots and residual-versus-fitted plots. If assumptions are violated, alternative transformations or analyses on the raw scale may be considered.

Results will be presented as estimated marginal means with standard errors, 95% confidence intervals, and nominal p-values for all three pairwise comparisons, consistent with the blinded analysis approach described in Section 10.1.

10.2 Secondary Efficacy Analyses

The secondary endpoint is the percentage change in P1NP from baseline to week 21. Following the strategy for the primary endpoint, this will be analysed using an MMRM as the primary approach, with an ANCOVA model used for sensitivity analysis.

Primary Analysis (MMRM): The MMRM will include all available post-baseline P1NP measurements (weeks 5, 13, and 21).

- **Outcome:** Log-transformed P1NP.
- **Fixed effects:** Treatment group, time (categorical), and treatment × time interaction.
- **Covariates:** Log-transformed baseline P1NP, age, and sex.
- **Specifications:** Unstructured covariance, REML estimation, and Kenward–Roger approximation.

Sensitivity Analysis (ANCOVA): To assess the robustness of the results, an ANCOVA will be performed on the percentage change at week 21.

- **Fixed effect:** Treatment group.
- **Covariates:** Baseline P1NP, age, and sex.
- **Imputation:** Missing week 21 values will be handled via Last Observation Carried Forward (LOCF) using the last post-baseline measurement.

Results will be reported as estimated marginal means, standard errors, and two-sided 95% confidence intervals. Consistent with the approach described in Section 10.1, all three pairwise comparisons will be reported. No multiplicity adjustments will be applied to the secondary analyses.

10.3 Subgroup analyses

The following pre-specified exploratory subgroup analyses may be performed by adding a treatment × subgroup interaction term to the primary MMRM model. All subgroup analyses are hypothesis-generating, will not be adjusted for multiplicity, and results will be interpreted descriptively.

Subgroups, defined in Section 8.3, include:

- Senescent cell burden: (highest tertile versus lower two tertiles p16INK4a)
- Baseline BMD category: (osteopenia vs osteoporosis)
- Renal function category: (eGFR strata)
- Age group: (<75 / ≥75 years)
- Sex: (male versus female)
- BMI category: (<25 [normal], 25–29.9 [overweight], ≥30 [obese])
- Smoking status: (current, former, never)
- Physical activity level: (Low, Moderate, High based on daily activity)

Subgroup results will be presented descriptively using interaction p-values and forest plots.

10.4 Planned exploratory efficacy analyses

10.4.1 Exploratory biochemical markers

Additional bone turnover markers collected (e.g., osteocalcin, BAP, TRAcP) will be summarised descriptively at baseline, week 5, week 13, and week 21. No formal hypothesis testing is planned.

10.4.2 DXA-derived outcomes

Lumbar spine, total hip, and femoral neck BMD will be summarised at baseline and week 21. Exploratory ANCOVA models adjusting for baseline BMD may be used.

10.4.3 HR-pQCT-derived outcomes

The following HR-pQCT parameters will be summarised at baseline and week 21:

- Total volumetric BMD
- Cortical thickness
- Cortical porosity
- Trabecular bone volume fraction
- Trabecular thickness
- FEA-derived failure load

Exploratory ANCOVA may be applied to estimate between-group differences.

10.4.4 Senescence and molecular markers

SASP factors, p16INK4a expression, and senescent cell quantification in bone outcomes will be summarised descriptively. These analyses are mechanistic and not powered for formal comparisons.

10.4.5 Physical function and body composition

Outcomes including grip strength, 30-second Sit-to-Stand, Timed-Up-and-Go, and DXA-derived body composition measures (lean mass, fat mass, android/visceral fat distribution) will be summarised descriptively. Exploratory ANCOVA may be applied where appropriate.

10.5 Adverse Events

Adverse events (AEs) will be summarised using the Safety Population. Frequencies and percentages will be reported by treatment group and system organ class. Between-group comparisons may be conducted using Fisher’s exact test or chi-square tests. AEs will not be imputed.

10.6 Clinical Laboratory Evaluations

Laboratory parameters (e.g., haematology, renal function, liver enzymes) will be summarised descriptively at baseline and scheduled visits. No formal hypothesis testing is planned.

11 Reporting Conventions

P-values ≥ 0.001 will be reported to three decimal places, and p-values < 0.001 will be reported as "< 0.001". Means, standard deviations, and other summary statistics (excluding quantiles) will be presented with one additional decimal place relative to the original measurement scale.
Quantile-based statistics, including medians and minimum/maximum values, will be reported using the same number of decimal places as the original data.
Any deviations from the statistical methods or reporting principles outlined in this Statistical Analysis Plan will be documented and justified in the final study report.

12 Listing of Tables, Figures and Listings

The following tables and figures will be prepared to summarise participant characteristics and study outcomes. Each table and figure will present results by treatment group in the order **D+Q, NR, Control**, and will be clearly annotated with the analysis population and any applicable footnotes.

12.1 Tables

- **Table 1:** Baseline characteristics (demographics, clinical variables, baseline CTX, P1NP, BMD, HR-pQCT variables, physical function, and body composition).
- **Table 2:** Summary of primary and secondary endpoints (percentage change in CTX and P1NP at week 21).
- **Table 3:** Exploratory biochemical endpoints (descriptive summaries of additional bone turnover markers at baseline, week 5, week 13, and week 21).
- **Table 4:** DXA-derived outcomes at baseline and week 21 (lumbar spine, total hip, femoral neck BMD; whole-body composition variables).
- **Table 5:** HR-pQCT-derived outcomes at baseline and week 21 (total vBMD, Ct.Th, Ct.Po, Tb.Th, Tb.BV/TV, FEA).
- **Table 6:** Summary of adverse events by treatment group.
- **Table 7:** Summary of clinical laboratory parameters across study visits.

12.2 Figures

- **Figure 1:** CONSORT flow diagram of participant disposition from screening to week 21.

- **Figure 2:** Longitudinal CTX trajectories (baseline, week 5, week 13, week 21) by treatment group.
- **Figure 3:** Longitudinal P1NP trajectories (baseline, week 5, week 13, week 21) by treatment group.
- **Figure 4:** Change in BMD (lumbar spine, total hip, femoral neck) from baseline to week 21.
- **Figure 5:** Change in HR-pQCT variables from baseline to week 21 (as appropriate for each parameter).
- **Figure 6:** Exploratory analyses of SASP factors and bone senescence markers (descriptive visualisation as relevant).
- **Figure 7:** Summary of physical function outcomes (baseline and week 21).
- **Figure 8:** Summary of body composition changes (baseline to week 21).

12.3 Listings

Additional data listings (e.g. participant-level raw values, protocol deviations, adverse events) will be produced as required for regulatory submission or internal review and will follow the same population and labelling conventions.