Statistical Analysis Plan

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1 SAP Signatures

I give my approval for the attached SAP entitled Targeting mTOR with everolimus and/or physical training for preventing postmenopausal bone loss and accelerated skeletal aging. The RapaLoad study, dated March 18th 2026.

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3 Abbreviations and Definitions

AE	Adverse Event
AR	Adverse Reaction
CRF	Case Report Form
P1NP	Procollagen type 1 N-terminal Propeptide
CTX	C-terminal telopeptide of type 1 collagen
TRAcP	Tartrate resistant acid phosphatase
BMD	Bone mineral density
VO_2max	Maximal Oxygen Uptake
SASP	Senescence Associated Secretory Phenotype
mTOR	Mechanistic target of Rapamycin
SF-12	Short form 12
DXA	Dual X-ray absorptiometry
HRpQCT	High-resolution peripheral quantitative computed tomography
LH	Luteinizing Hormone
FSH	Follicle-stimulating hormone
SAP	Statistical Analysis Plan
FDA	Food and drug administration (US)
PCS-12	Physical Component Summary-12
MCS-12	Mental Component Summery-12

4 Introduction

4.1 Preface

It is estimated that women lose around 20-25% of bone mass during the 5-10 years period of postmenopausal transition, and menopause is considered a major risk factor for osteoporotic fragility fracture. Currently, there is no preventive strategy to counteract these changes, and hormone replacement therapy is not considered an attractive option for many women, because of concerns related to side effects. Rapamycin, through inhibition of mTOR (the mechanistic target of Rapamycin) has been demonstrated in many preclinical animal models to extend lifespan and healthspan. The most well-established mTOR inhibitors include rapamycin (sirolimus) and its analog (also referred to as rapalog). Everolimus are FDA approved and have been used for many years at a high dose as an immunosuppressant and anti-cancer drug. Exercise training has been demonstrated to counteract age-related degenerative changes in several clinical studies. The aim of this randomized clinical trial is to test the effects of treatment with oral Everolimus, exercise, or their combination as a preventive strategy for impaired musculoskeletal function in healthy postmenopausal women.

4.2 Scope of the analyses

In this trial, the efficacy of 3 intervention strategies for 24 weeks in comparison to a control

group receiving no specific recommendation are tested and followed by pair wise comparisons. Interventions are: 1/ Football Fitness training twice weekly for 1h, 2/ Everolimus once weekly and 3/ Everolimus and Football Fitness. The analysis will assess their effects on several outcomes including changes in bone turnover markers in the blood, bone mineral density by dual x-ray absorptiometry (DXA), bone strength and microarchitecture by HR-pQCT, muscle function and postural balance by physical evaluation, cardiopulmonary function by VO_2max measurements and metabolic health.

5 Study Objectives and Endpoints

5.1 Study Objectives

This study aims to determine if treatment with everolimus, exercise training, or their combination for 24 weeks enhances bone formation in healthy postmenopausal women.

5.2 Endpoints

Primary endpoint

-Percentage change in circulating levels of bone formation marker N-terminal fragment of procollagen type 1 (P1NP) at 24 weeks as compared with baseline.

Secondary endpoints

Change in circulating levels of bone turnover markers:

-Bone resorption markers (C-terminal telopeptide of type 1 collagen (CTX) and Tartrate resistant acid phosphatase (TRAcP)) at baseline, 2, 4, 12 and 24 weeks.

-Bone formation markers (osteocalcin, and bone alkaline phosphatase) at baseline, 2, 4, 12 and 24 weeks and P1NP at 2, 4 and 12 weeks.

-Lumbar spine (L1-4), and total hip and femoral neck bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DXA) at baseline and 24 weeks.

-Bone microarchitecture, mass, and geometry at the distal radius and tibia assessed using high-resolution peripheral quantitative computed tomography (HR-pQCT) at baseline and 24 weeks.

-Muscle function and postural balance tested as previously described [44, 45] at baseline and 24 weeks

-Cardiopulmonary health estimated by measuring VO_2max at baseline and week 24.

-Metabolic health: weight, body composition by DXA scanning (muscle mass and fat mass), fasting blood glucose, fasting insulin, lipid parameters and metabolomic studies at baseline and week 24.

Exploratory analyses are planned to address:

- Impact of the different interventions and their combination on aging clocks as determined by epigenetic clocks established based on DNA methylation pattern.
- Understanding the role of those interventions on the immune system composition.
- Evaluation of senescence burden as evaluated by serum levels measurement of senescence associated secretory phenotype (SASP).

- Quality of life questionnaire SF-12.
- From a subgroup of at least 10 participants in each intervention group we will address cellular and molecular impact of Everolimus or football fitness or their combination on bone materials properties (bone biopsies) and on bone marrow mesenchymal stem cells properties and capacities.

6 Study Methods

6.1 General Study Design and Plan

This is a 24-weeks phase 2, randomized trial of the effect of everolimus, physical training and the combination of both on bone and muscle health in 136 healthy postmenopausal women aged 45-60 years.

6.2 Inclusion-Exclusion Criteria and General Study Population

Inclusion criteria

- Postmenopausal women aged 45-60 years old as evidenced by measuring serum levels of LH and FSH and absence of menstruation for at least 1 year.
- No history of low energy hip or vertebral fractures during the last 6 months.
- Ability to provide informed consent.

Exclusion criteria

- Diabetes (type 1 and 2).
- Heart failure similar to NYHA Class IV.
- Primary hyperparathyroidism.
- Known disorders affecting bone metabolism, e.g., uncontrolled thyrotoxicosis, severe renal impairment (eGFR <20) or liver function (baseline phosphatase higher than twice upper limit (105 U/L)), active rheumatic diseases, celiac disease, severe chronic obstructive lung disease (COPD), hypopituitarism, or Cushing's disease.
- Previous use of bone antiresorptive or bone anabolic drugs within the last 5 years.
- Use of anabolic steroids in the previous year.
- Subjects who require treatment with strong CYP3A4 inhibitors or inducers.
- History of coagulopathy or medical condition requiring long-term anticoagulation.
- Anemia – Hg < 9.0 g/dl, Leukopenia – white blood cells (WBC) < 3,500/mm^3, Neutropenia absolute neutrophil count < 2,000/mm^3, or Platelet count – platelet count < 125,000/mm^3.
- Patients with impaired wound healing or history of a chronic open wound.
- Scheduled for immunosuppressant therapy for transplant or scheduled to undergo chemotherapy or any other treatment for malignancy.
- Untreated dyslipidemia with LDL-c > 4.9 mmol/L and family history of dyslipidemia, Total cholesterol > 9.1 mmol/L, or triglycerides > 9.9 mmol/L.
- Any form of clinically relevant primary or secondary immune dysfunction or deficiency.

- Unstable ischemic heart disease.
- Bone mineral density (BMD) measured by DXA scanning with T-score <-3.
- Known allergy to rapamycin or rapalogs.
- The study will exclude participants with inability to speak and understand Danish and with inability to cooperate or perform physical training.
- Inability to give informed consent.

6.3 Randomization

A stratified randomization will be performed to a total of 136 participants to one of the 4 below-mentioned interventions. Each participant is assigned a subject number when consent is obtained, inclusion and exclusion criteria have been reviewed, and the participant otherwise meets the requirements for study participation and continues to participate in the study. A participant cannot be assigned more than one randomization number, and this number cannot be used for other persons. Randomization will be performed using the RedCAP® module provided by Odense Patient data Explorative Network (OPEN) at Odense University Hospital (OUH). The stratification will be performed according to years post menopause. We will use only 2 strata within 5 years post menopause or more than 5 years and up to 10 years.
Participants will be randomized to one of the 4 groups (34 individuals in each group). The interventions in each group are as follow:

- (1) Control group: asked to continue their life as usual with no specific recommendation.
- (2) Football fitness group: structured hybrid exercise training via the "football fitness concept" organized as a supervised group training at the University of Southern Denmark, Department of Sports Science and Clinical Biomechanics. "Football Fitness concept" is designed for participants with little prior experience with football and it is multifaceted training incorporating endurance training, high-intensity interval training (HIIT) and strength training. The training session lasts 60 minutes, and participants will receive two training sessions per week for 24 weeks. Each session is composed of 4 elements: a 15 min warm-up that includes strength and balance exercises, a 15 min period with technical pair-based drills, and a 30-min period with small-sided football drills [32]. Training will start once a group of 6 persons is formed and 3 training sessions per week will be offered, allowing flexibility and increasing compliance.
- (3) Everolimus group: receiving an oral dose of everolimus 5 mg once a week for 24 weeks.
- (4) Everolimus and Football Fitness group: everolimus as described in group #3, with Football training session as described in group #2 for 24 weeks.

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Table 1. Study overview

Visit nr.	**0**	**1**	**2**	**3**	**3[1]**	**4**	**4[2]**	**5**	**6**	**7**	**8**	**9**
Week	-1	0	0	0	Start	2	2	4	12	24	24	50

Place	IOB	IOB	IOB	OUH	At home	OUH	Telephone	OUH	OUH	IOB	OUH	IOB
Estimated duration (in hours)	1	2-3	2	2-3		1	0,25	1	1	6	2-3 (evt. + 1[3])	2-3
Information conversation	x											
Consent		x										
Baseline number		x										
Reviewing of inclusion and exclusion criteria		x	X									
Randomization			X									
Health information		x										
Seismofit		x								x		
Blood pressure, pulse, temperature and evt. Objective examination		x						x	x	x		
First intake of medicin					x							
Side effects checking							X	x	x	x		x
Blood sample screening		x										
Fasting blood samples				x		x		x	x		x	x
InBody 270		x								x		
DXA-scanning		x								x		x
HR-pQCT scanning				x							x	
Fitness test evaluation			x							x		
Dispensing and reviewing medication				x								

and side effects diary												
Dispensing of investigational medication				x								
Quality of life questionnaire (SF-12)			x							x		
Bone biopsies and bone marrow aspirates											x	
Final visit										x	x	
Follow up visit												x

6.4 Study Assessments

Table2. Analysis Time Windows

Visit (target day)	Lower bound (days)	Upper bound (days)
Baseline (0)	N/A	N/A
Randomization (Day 1)	N/A	56 days after baseline visit
Week 2 (14)	10	18
Week 4 (28)	20	36
Week 12 (84)	70	98
Week 24 (168)	154	182
Week 50 (350)	336	364

All the outcomes below are measured as values at baseline/randomization compared to T=24 weeks (for bone turnover markers values have also been measured at T=0.5, 1, 3, 6, and 12 months after randomization (except analyses on bone biopsies as these are only collected at T=24 weeks)

- **Bone turnover markers**
- **P1NP**
  - Marker for bone formation. Measured in blood ranging between 23-125 µg/l in postmenopausal women.
- **CTX**

  - Marker for bone resorption. Measured in blood ranging between 0.18-1.02 µg/l in postmenopausal women.
- **TRAcP**
  - Marker for bone resorption ranging between 3,29+-1,07U/L versus 1.70+-0.59U/L according to assays
- **Osteocalcin**
  - Postmenopausale women 3.9 -21.6 ng/mL
- **Bone alkaline Phosphatase**
  - Adult women 12-56.7U/L
- **Bone geometry and microarchitecture measured by HRpQCT**
  - Total volumetric bone density (Tot.vBMD) in mg HA/cm^3.
  - Cortical volumetric bone density (Co.vBMD) in mg HA/cm^3.
  - Trabecular volumetric bone density (Tb.vBMD) in mg HA/cm^3.
  - Total Area (mm^2).
  - Cortical thickness (CT.Th) (mm).
  - Cortical porosity (Ct.Po) (%).
  - Trabecular bone volume per tissue volume (Tb. BV/TV) (%).
  - Trabecular number (Tb.Th) (1/mm).
  - Trabecular thickness (Tb.Th) (mm).
- **Bone strength measured using HR-pQCT by Finite Element Analysis (FEA)**
  - Failure load = estimated energy needed to fracture. Measured in N (Newton).
  - Bone stiffness.
- **Questionnaire:**
  Quality of life measured by the Short Form Health Survey (SF-12)

Anthropometric measurements
Height (cm)
Waist circumference (cm)
Hip circumference (cm)

InBody
Weight (kg)
Muscle mass (kg)
Fat mass (procent)
Fat mass (kg)
BMI (kg/m^2)

DEXA scanning
T-score L1-L4
T-score L2-L4
T-score femoral neck, right leg
T-score femoral neck, left leg

T-score total hip, right leg
T-score total hip, left leg
T-score whole body

Total Legs BMD (g/cm^2)
Left Leg BMD (g/cm^2)
Right Leg BMD (g/cm^2)
Total Arms BMD (g/cm^2)
Left Arm BMD (g/cm^2)
Right Arm BMD (g/cm^2)
Body BMD (g/cm^2)

Total Legs Fat (%)
Left Leg Fat (%)
Right Leg Fat (%)
Total Arms Fat (%)
Left Arm Fat (%)
Right Arm Fat (%)
Body Fat (%)

Total Legs Fat Free Mass (gr)
Left Leg Fat Free Mass (gr)
Right Leg Fat Free Mass (gr)
Total Arms Fat Free Mass (gr)
Left Arm Fat Free Mass (gr)
Right Arm Fat Free Mass (gr)
Body Fat Free Mass (gr)

Total Legs BMC (gr)
Left Leg BMC (gr)
Right Leg BMC (gr)
Total Arms BMC (gr)
Left Arm BMC (gr)
Right Arm BMC (gr)
Body BMC (gr)

Total Legs Fat mass (gr)
Left Leg Fat mass (gr)
Right Leg Fat mass (gr)
Total Arms Fat mass (gr)
Left Arm Fat mass (gr)
Right Arm Fat mass (gr)
Body fat mass (gr)

Seismofit measurement

VO_2max (ml*kg^{-1}*min^{-1})
Resting heart rate (bpm)
Respiratory rate (breaths)
Temperature (celsius)

Resting heart rate and blood pressure
Systolic blood pressure, right arm (mmHg)
Diastolic blood pressure, right arm (mmHg)
Calculated Mean arterial pressure, right arm (mmHg)
Resting heart rate, right arm (bpm)

Systolic blood pressure, left arm (mmHg)
Diastolic blood pressure, left arm (mmHg)
Calculated Mean arterial pressure, left arm (mmHg)
Resting heart rate, left arm (bpm)

Football fitness training
Attendance, number of sessions

Fitness tests

Y-balance
Anterior direction, standing on right leg (cm)
Postmedial direction, standing on right leg (cm)
Postlateral direction, standing on right leg (cm)

Anterior direction, standing on left leg (cm)
Postmedial direction, standing on left leg (cm)
Postlateral direction, standing on left leg (cm)

Stork balance
Standing on right leg (s)
Standing on left leg (s)

Sit-to-stand chair test
Max fully completed stands (n)

Handgrip strength test (right/left)
Max grip strength (kg)

Power rig (right/left)
Max Power (W)
Watts per kg (W/kg)

Counter Movement Jump
Vertical Jump height [JH] (cm)

Vertical jump height relative to ground (cm) [calculated as JH + BCM position at toe-off]
Vertical BCM velocity at toe-off (m/s)
Time at onset of concentric phase (ms)
Time at onset of eccentric deceleration phase (ms)
Time at onset of integration [onset ECC phase] (ms)
Peak concentric Power [Ppeak] (W, W/kg))
Time at Ppeak (ms)
Time at Ppeak relative to onset of concentric phase (ms)
BCM Position at onset of concentric phase (cm)
BCM Position at onset of ECC deceleration phase (cm)
BCM Position at Ppeak (cm)
BCM position at toe-off (cm)
Mean concentric Power (W, W/kg)
Work concentric phase (J, J/kg)
Work eccentric phase (J, J/kg)
Mean eccentric Power (W, W/kg)
Peak eccentric power* (W, W/kg) * OBS peak *negative* ECC power
Rate of force Development RFD (0–50 ms relative to onset of ECC dec phase) ($N \cdot s^{-1}$, $N \cdot s^{-1}$/kg)
Lower Limb Stiffness (kN/m, kN/m/kg)
Duration of concentric phase: Tcon (ms)
Duration of eccentric deceleration phase: Tecc-dec (ms)
Peak vertical Fz force CONcentric phase (N, N/kg)
Peak vertical Fz force ECCcentric phase (N, N/kg)
Time at Peak vertical Fz force CONcentric phase (ms)
Time at Peak vertical Fz force ECCcentric phase (ms)
Mean vertical Fz force CONcentric phase (N, N/kg)
Mean vertical Fz force ECCcentric phase (N, N/kg)

Isometric leg press (right/left)

Peak Isometric Force (N, N/kg)
Time at Peak Isometric Force (ms)
Baseline force level (N)
Time at onset of force (ms)
Force at onset of force (N)
Rate of force Development RFD (0–30 ms) ($N \cdot s^{-1}$, $N \cdot s^{-1}$/kg)
Rate of force Development RFD (0–50 ms) ($N \cdot s^{-1}$, $N \cdot s^{-1}$/kg)
Rate of force Development RFD (0–100 ms) ($N \cdot s^{-1}$, $N \cdot s^{-1}$/kg)
Rate of force Development RFD (0–200 ms) ($N \cdot s^{-1}$, $N \cdot s^{-1}$/kg)
Impulse (0–30 ms) (N·s, N·s/kg)
Impulse (0–50 ms) (N·s, N·s/kg)
Impulse (0–100 ms) (N·s, N·s/kg)
Impulse (0–200 ms) (N·s, N·s/kg)

Stairs climb test (9-stairs)

Time for climbing 9 stairs in sec.

6-min walking test
Walk distance (m)
Heart rate average (bpm)
Heart rate max (bpm)
Rating of perceived exertion (RPE – Borg scale)

Bicycle ergometer test
Height (cm)
Weight (kg)

Submax 1 (0-5 min)
VO_2 submax 1 (ml/min)
VO_2/kg submax 1 (ml/kg/min)
RER submax 1
Heart rate averages submax 1 (bpm)

Submax 2 (5-8 min)
VO_2 submax 2 (ml/min)
VO_2/kg submax 2 (ml/kg/min)
RER submax 2
Heart rate averages submax 2 (bpm)

Maxtest (8 – max min)
VO_2max (ml/min)
VO_2max/kg (ml/kg/min)
RER peak
Heart rate max (bpm)
Watt max (watt)
Time To Exhaustion (min:s)
Rating of perceived exertion (RPE – Borg skala)

Metrics from trainings (Kinexon-Polar-Next 11)
Kinexon
Distance (m)
Distance / min (m)
Distance (speed | 0-2) (m)
Distance (speed | 2-5) (m)
Distance (speed | 5-9) (m)
Distance (speed | 9-13) (m)
Distance (speed | 13-16) (m)
Distance (speed | 16-20) (m)
Distance (speed | >20) (m)
High Metabolic Power Distance (m)
Metabolic Power (max.) (W)

High Speed and Acceleration Distance (m)
Speed (Ø) (km/h)
Speed (max.) (km/h)
Speed (% of max.) (%)
Sprints (>5)
Sprints (0-2)
Sprints (2-5)
Sprints (5-9)
Sprints (9-13)
Sprints (13-16)
Sprints (16-20)
Sprints (>20)
Total Accelerations
Intense Accelerations
Accelerations (0-1)
Accelerations (1-1.5)
Accelerations (1.5-2)
Accelerations (2-2.5)
Accelerations (≥ 2.5 m/s^2)
Acceleration (max.) (m/s^2)
Accumulated Acceleration Load
Total Decelerations
Intense Decelerations
Decelerations (0-1)
Decelerations (1-1.5)
Decelerations (1.5-2)
Decelerations (2-2.5)
Decelerations (≥ 2.5 m/s^2)
Deceleration (max.) (m/s^2)
Accumulated Deceleration Load

Polar
HR avg [bpm]
HR-max [bpm]
HR avg [%]
HR-max [%]
Time in HR-zone 1 (50 - 59 %)
Time in HR-zone 2 (60 - 69 %)
Time in HR-zone 3 (70 - 79 %)
Time in HR-zone 4 (80 - 89 %)
Time in HR-zone 5 (90 - 100 %)
Min RR interval
Avg RR interval
Max RR interval

Next 11

Player Load Right
Player Load Left
Load/Min Right
Load/Min Left
Explosive [seconds] Right
Explosive [seconds] Left
Explosive Actions Right
Explosive Actions Left
Explosive Movements Right
Explosive Movements Left
Explosive Movements [seconds] Right
Explosive Movements [seconds] Left
Very High [seconds] Right
Very High [seconds] Left
Very High Actions Right
Very High Actions Left
Very High Movements Right
Very High Movements Left
Very High Movements [seconds] Right
Very High Movements [seconds] Left
High [seconds] Right
High [seconds] Left
High Actions Right
High Actions Left
High Movements Right
High Movements Left
High Movements [seconds] Right
High Movements [seconds] Left
Moderate [seconds] Right
Moderate [seconds] Left
Moderate Actions Right
Moderate Actions Left
Low [seconds] Right
Low [seconds] Left
Low Actions Right
Low Actions Left

Some of the anthropometric and physiological outcomes will be reported in separate publications after the primary publication.

7 Sample Size

There are no previously reported studies of the effect of everolimus on bone metabolism. The primary effect is the intention-to-treat relative difference in change in P1NP at 24 weeks. Based on a similar 20-week RCT in postmenopausal women treated with beta-blockers [41], the sample size was calculated to compare the percentage changes in P1NP

from baseline to 24 weeks be-tween everolimus and control groups. In order to achieve statistical significance, the study aims to enroll a total of 34 participants in each group. With this sample size, the study would possess 80% statistical power to detect a 20% difference in P1NP. The power calculation was based on a two-sample t-test, utilizing a two-sided significance level of 0.05. This calculation also considers an estimated withdrawal rate of 10% for both groups, which is deemed realistic due to the low incidence of adverse events typically observed with everolimus at this dosage. We have the assumption that the difference in P1NP level between 24 weeks and baseline will be larger in the exercise training (FTS) group based on the previous publication of our collaborator Peter Krustrup [51, 52]. We also assume that the changes observed in the group with everolimus and FTS will be larger as we expect an enhanced effect of the drug when combined with FTS.

8 General Analysis Considerations

8.1 Timing of Analyses

The main analysis will be performed when all subjects have completed the 24-week intervention last visit (Visit 8, week 24). Data will be transferred to the file “RapaLoad Analyses” after last patient last visit and after the finalization and approval of this SAP document. The 50 weeks endpoint will be analyzed when all subjects have completed their Visit 9- 50 weeks.

8.2 Analysis Populations

8.2.1 Full Analysis Population (Intention to Treat)

- All subjects who were randomized independent of treatment dose.

8.2.2 Analysis Population (per protocol)

- Only randomized subjects, having performed at least 70% of the Football Fitness protocol, would be analysis for a per protocol analysis (i.e. ≥33 training sessions).

8.2.3 Safety Population

- All subjects who received any study treatment (including control) and are confirmed as providing complete follow-up regarding adverse event information.

8.3 Subgroups

A stratification has been employed to subgroup the participants of this trial according to years post menopause. Participants were stratified before randomization using 2 strata within 5 years post menopause or more than 5 years and up to 10 years.

8.4 Missing Data

Data are collected and managed using REDCap. Missing data are represented as blank fields in the database.

8.5 Multiple Testing

No multiple testing will be performed.

9 Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics by randomization group: n (non-missing sample size), median with interquartile range. Categorical variables are summarized by n (non-missing sample size). A summary table will be constructed with a column for each intervention in the order (Control, Fitness Football, Everolimus and Everolimus and Fitness Football) and will be annotated with the total population size relevant to that table/intervention, including any missing observations.

9.1 Subject Disposition

This will include the number screened, excluded, randomized with an overview of time-dependent rates of recruitment using a skeleton CONSORT flow diagram.

9.2 Derived variables

P1NP
CTX
Tot.vBMD
CT.Th
Ct.Po
Tb. BV/TV
Tb.Th
FEA
BMI
Lumbar BMD
Total hip BMD
Total body fat
Total body lean mass
From Sit to stand chair test: STS mean power ($W \cdot kg^{-1}$)
From Handgrip strength test: Max grip strength (N)
From Stair climb test (9 stairs): Power (Watt)
Physical Component Summery-12 (PCS-12)
Mental Component Summery12 (MCS-12)

9.3 Demographic and Baseline Variables

Age
Ethnicity
Weight

Height
Body mass index
Osteopenia, T score
Biochemistry

- FSH
- LH
- Estradiol
- CBC (Red blood cells, Leucocytes, total neutrophiles, platelets, haemoglobin, hematocrit)
- Sodium
- Potassium
- Creatinine
- eGFR
- ionized calcium
- parathyroid hormone
- 25-OH Vitamin D3
- Liver transaminase (ALAT)
- Alkaline phosphatase (ALP)
- Albumin (ALB)
- Coagulation factors (KFNT/PP)
- TSH
- T4
- CRP
- LDL-c
- HDL-c
- Triglycerides
- Total cholesterol
- HbA1C

DXA

- Hip BMD
- Lumbar spine BMD
- Total fat body mass
- Total lean body mass

Blood pressure
Heart rate
Respiratory rate
Temperature

9.4 Intervention Compliance

- Football Fitness session compliance was checked by registration of participation in football fitness sessions by trainers.
- Compliance to Everolimus intervention was checked by inspecting the drug remains collected at endpoint or when participants ended their participation in the trial.
- Each participant was given a diary at the start of the trial where they had to report

on compliance. Intervention compliance was mandatory to ask participants about at all visits.

10 Efficacy Analyses

Primary and secondary efficacy analyses are performed on an intention-to-treat basis as detailed below.

10.1 Primary Efficacy Analysis

Multiple linear mixed model. This is implemented as a mixed model for repeated measures (MMRM) on all follow-up time points using an unstructured marginal covariance matrix on the participant level. The outcome is P1NP. The systematic part of the model consists of treatment (a factor/class variable) and time (factor variable) and the treatment-time interaction. The model further adjusts known prognostic variables: age and time from menopause. The outcome is analysed on logarithmic scale.

The model is fit by restricted maximum likelihood. Confidence intervals and tests employ the Kenward-Roger approximation by its correction to the standard error matrix of the fixed effects and associated degrees of freedom.

If convergence issues are observed, the analyst may increase the number of iterations used to find starting values and/or change the maximization algorithm. If this does not ameliorate the issue, a simpler covariance structure will be used with homoscedasticity at all time points and serial correlations decaying at an exponential rate. If convergence does not ensue, a random intercept model will be used implying compound symmetry of the marginal covariance matrix.

Model validation will be performed by visual inspection of QQ-plots of standardized residuals and possibly random effect estimates (BLUPs) along with plots of standardized residual vs fitted values to assess variance homoscedasticity. If assumptions are deemed to be violated the analyst may change between the original and logarithmic scale. If this does not solve the issue, results will be reported on the scale where they are most easily interpreted and potential issues with model assumptions will be reported.

10.2 Secondary Efficacy Analyses

Efficacy analyses for secondary outcome variables are performed in the same manner as for the primary outcome. When there is only one follow-up measurement, the proposed analysis coincides with the ANCOVA model.

10.3 Subgroup analyses

The primary analysis will be repeated for subgroups defined by age, time from menopause as defined in our stratification plan (<5 years from menopause or > 5 years), and side effects (y/n). As mentioned in section 8.2.2 Per protocol analysis will be performed as well in the stratified groups.

Each subgroup analysis is performed by modifying the model described in Section 10.1 so that intervention is replaced by the interaction between intervention and the appropriate subgroup variable. The baseline to 24 weeks effect between groups is reported for each subgroup along with an estimate of the difference between subgroups.

For side effects it is realized that the subgroup analysis involves stratifying on a post-randomisation variable and the associated analyses will be interpreted cautiously.

10.4 Planned exploratory efficacy analyses

Explorative analyses will take place later point and an appendix will be submitted later on for that.

10.5 Adverse Events and Adverse Reactions

All Adverse Events (AEs) and Adverse Reactions (ARs) will be collected and reported in REDCap. For each AE and AR, the start date and end date will be recorded, as well as management of the event (whether the intervention was continued, paused, modified, or discontinued). If the AE or AR is ongoing, it will be reported in the database.
For AEs, the investigator will assess whether there is a potential relationship to the study intervention. For ARs, the investigator will assess whether the event is due to the everolimus intervention, the football fitness intervention, or combination of both interventions.
All AEs and ARs will be evaluated for seriousness. An AE that meets seriousness criteria will be classified as a Serious Adverse Event (SAE) and an AR that meets seriousness criteria will be classified as a Serious Adverse Reaction (SAR).
At each visit, participants will be asked if they have experienced any discomfort or injuries, and this will be registered in REDCap.
If an AE or AR is ongoing at the end-point visit (visit 7), it will be reported in REDCap and will be followed up at visit 9. Everolimus has a half-life (T½) of 15-35 hours and is expected to be eliminated by the end-point visit.

10.6 Clinical Laboratory Evaluations

The summary statistics will be produced in accordance with section 9.

11 Reporting Conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as “<0.001”. The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data.

Any deviations from the statistical principles and procedures described in this SAP

document will be reported with justification.

12 Listing of Tables, Listings and Figures

This section is to give precise details for each table, listing or figure to be produced.

Figure 1: Flowchart of inclusion of study participants
Table 1: Baseline characteristics. Includes headings “Characteristics” “Control “Football Fitness Session” “Everolimus” and “Everolimus+ Football Fitness Session”
Figure 2: Overall study design, Changes in P1NP over time. X-axis = weeks (0, 2, 4, 12, and 24 weeks); Y-axis 1 = µg/L , Changes in CTX over time. X-axis = weeks (0, 2, 4, 12, and 24 weeks); Y-axis 1 = ng/L, time course of changes for BTMs .
Table 2: numerical values (bone outcomes)

- BTM (also TRAcP and osteocalcin and BALP
- DEXA scan, BMD/BMC. Total/ different sites
- HRpQCT microarchitecture/ µFinite Element Analysis
- Bone biopsies: measurements

Figure 3: Forrest plot to visualize effect of everolimus, exercise or their combination on bone outcomes and bone biopsies photos
Table 3: Fitness outcomes

- Body composition (BMI, lean, fat percentage, WHR
- Glucose metabolism / cholesterol /LDL HDL..
- VO2max/ heart rate/ respiratory rate/ blood pressure
- Muscle fitness (Y balance, stork balance7 sit to stand/ Hand grip/ power rig/ counter mov. Jump/ isometric leg press
- Q of life SF.12 (PCS-12 and MCS-12)

Figure 4: Forrest plot to visualize effect of everolimus, exercise or their combination on fitness outcomes and correlations between bone and fitness outcomes.
Table 4: safety profile/ adverse events