Treatment of Primary Hyperparathyroidism with Denosumab and Cinacalcet.

EudraCT number: 2016-001510-20

NTC: 03027557

Sponsor Protocol number: 180987

Subinvestigator:

Julius Simoni Leere, Cand. Med.

Registrar, Region Nordjylland

Autorisation-ID: 0BKC2

Sponsor and Investigator:

Peter Vestergaard, MD, Professor, Ph.d., Dr.med.

Endokrinologisk Afdeling, Aalborg Universitetshospital, Mølleparkvej 4,

9000 Aalborg

Single-center study.

Studyprotocol

Treating Primary Hyperparathyroidism with Denosumab and Cinacalcet.

1. Generel provisions.

Trialcenter:

Endokrinologisk Afdeling, Aalborg Universitetshospital, Mølleparkvej 4, 9000 Aalborg,

Tlf. 97 66 36 00

Collaborationpartners:

Radiologisk Afdeling, Aalborg Universitetshospital, Mølleparkvej 4, 9000 Aalborg

Klinisk Biokemisk Afdeling, Aalborg Universitetshospital, Mølleparkvej 4, 9000 Aalborg

Sygehusapoteket, Aalborg Universitetshospital, Porsvej 1, 9000 Aalborg

GCP monitoring by:

The trial will be monitored by: GCP-enheden ved Aalborg og Aarhus Universitetshospitaler, Institut for Klinisk Medicin Aarhus Universitet, Brendstrupgårdsvej 100, 8200 Aarhus N.

Contact:

Forskningens Hus i Aalborg, Søndre Skovvej 15, 9000 Aalborg, Telephone: 9766 6266

The trial will be conducted according to the trial protocol, ICH-GCP guidelines and applicable legislation.

Date of trial initiation: 01-09-2016

Date of end of trial: 01-04-2019

Signature by sponsor and investigator:

Date: 26-04-16

Peter Vestergaard

Signature by Subinvestigator:

Date: 27-04-16 

Julius Simoni Leere

Table of contents

2. Background information.

List of abreviations is at the end of the document.

Background.

Primary Hyperparathyroidism (pHPT) is the most common disease in gl. parathyroidea, and the most common cause of hypercalcaemia. Today, approx. 900-1000 patients a year in Denmark. 1
The etiology is only partially elucidated, and there are several subtypes of the disease - both adenomas (85-90%), multiglandular hyperplasia, less common carcinomas and very rare hereditary mutations can give the diagnostic biochemical picture with high s-Ca2 + and at the same time elevated, or highly normal s-intact parathyroid hormone (s-iPTH). Common is a pathologically elevated secretion of PTH, which in turn directly and indirectly increases intestinal absorption, bone resorption and renal reabsorption of Ca2 +. 2
The disease expresses itself symptomatically very variable according to severity, and patients can debut with everything from pronounced hypercalcemic symptoms with severely affected general condition, to a completely asymptomatic random biochemically detected condition. The effects of pHPT are manifold and affect multiple organ systems. These include decalcification of the bones, which leads to osteoporosis and fractures, increased excretion of calcium from the kidneys, which leads to a negative calcium balance, as well as increased risk of nephrocalcinosis, formation of kidney stones. kidney failure. There is also an increased risk of cardiovascular disease, ulcer disease, pancreatitis and pancreatic insufficiency, confusion, memory loss, psychosis, joint and muscle pain and fatigue, etc. 2
More and more patients (80%) are diagnosed at an early (asymptomatic) stage by biochemical screening of s-calcium. Several studies indicate that this group of patients is also exposed to the effects of pHPT. Patients have increased bone remodeling3,4, which in turn has been shown by histomorphological analyzes, DXA and micro-CT to increase the cortical porosity of the bones and dilute the cortical width, while the trabecular bone thickness in mild pHPT may remain unaffected.5. The latter is controversial, however, as HRqCT analyzes show microstructural changes in the trabecular bone, which are considered to be able to lower its strength as well.6–10
Long-term studies suggest that the disease progresses over time, leading to an increase in scalcium and a decrease in BMD11,12. The increased bone turnover thus forms a rationale for treatment.
Whether the fracture risk in asymptomatic primary hyperparathyroidism is elevated is not yet fully elucidated, but cohort and population studies suggest that fracture risk is increased in both cortical and trabecular bone for up to 10 years before diagnosis and treatment of pHPT. A brand new prospective study of patients referred to a highly specialized center has shown that there is no difference in the incidence of vertebral fractures (VF), and densitometric osteoporosis between asymptomatic and symptomatic patients referred to a highly specialized center with pHPT (VFs, 34.4 vs 34.7%; osteoporosis at DXA, 59.4 vs 65.8%) 13.

Translation types

Text translation

Source text

1148 / 5000

Translation results

Curative treatment for pHPT is found today exclusively in the form of parathyroidectomy. Surgery is often not desirable in asymptomatic patients, just as a larger group of those diagnosed due to comorbidity, or for other reasons, meet the surgical criteria (found later in this document). However, over time, these patients are at high risk of developing complications of pHPT. An effective medical treatment alternative for those patients who are not surgery candidates would therefore be desirable. Thus, over time, several different solutions have been tried without finding an actual alternative to surgery. Medication is used today primarily in symptomatic patients while waiting for surgery, in severe symptomatic hypercalcaemia, or as a supplement to surgery e.g. in case of continued hypercalcaemia, or severe osteoporosis. Today, standard treatment for asymptomatic patients, often merely biochemical and radiological observation for signs of osteoporosis, renal involvement and calcium levels, is for the purpose of emergence of surgical indication. In the meantime, patients are often treated only with calcium and vitamin D.14

2.1 Drugs

The purpose of this study is to investigate whether treatment with Denosumab (ATC code: M05BX04) alone, or in combination with Cinacalcet (ATC code: H05BX01), may be an effective medical alternative for patients with low BMD pHPT who are not surgery candidates. Particular emphasis is placed on the treatment of hypercalcaemia, the prevention of osteoporosis and the effect of the treatment on bone structure and circulation. The medicine included in the trial is listed below. Cinacalcet and Denosumab will be the registered trial drug, and will be notified as such. Preparation name: Prolia® Generic Name: Denosumab ATC Code: M05BX04 Manufacturer: Amgen AB (Denmark). Strandvejen 70, 2900 Hellerup, Denmark Appearance: Green-white pen Dose: 1 syringe contains 60 mg (1 ml) of denosumab. Svt a dose. Description of the drug substance: Prolia is a recombinant human IgG2 monoclonal antibody with osteoclast inhibitory activity. The antibody is directed against RANKL. Decreased stimulation of the RANK receptor on preosteoclastic cells results in inhibited osteoclast formation and activity, thereby decreasing bone resorption, increasing bone mineral density, and reducing the risk of fracture. Rationale for dose use and duration of treatment: The usual dose for the treatment of osteoporosis is 60 mg x 1 every 6 months. V. prevention of fractures, and reduction of severe hypercalcemia in

malignant disease, doses as high as 120 mg at 8-day intervals may be used. For this study we will use: 60 mg, semi-annually for 1 year. Packing in the experiment: Experimental name. Subinvestigator's name. Randomization number. Lot number. The text "Prolia® / Placebo". Preparation name: Mimpara® Generic name: Cinacalcet ATC code: H05BX01 Manufacturer: Amgen AB (Denmark). Strandvejen 70, 2900 Hellerup, Denmark Method of administration: Oral, green oval tablets without notch. Dosage: 1 tablet (film-coated) contains 30 mg, 60 mg or 90 mg of cinacalcet (as hydrochloride). Description of the drug substance: Increases the sensitivity of the calcium-sensitive receptor (CaSR) to extracellular calcium, leading to decreased parathyroid hormone secretion. Rationale for dose use and duration of treatment: Treated for 1 year with 30 mg x 1 daily, for comparability with other clinical studies in the field, and to avoid the risk of hypocalcaemia. Packing for this experiment: Experiment name. Subinvestigator's name. Randomization number. Lot number. The text "Mimpara® / Placebo". Placebo 1 (placebo for prolia): Generic Name: Isotonic Saline (NaCl 0.9%), Fresenius Kabi, 10/20 ml vial. Manufacturer: Fresenius Kabi, Islands Brygge 57, 2300 København S ATC code: B05BB01, V07AB Method of administration: Subcutaneous, administered on the back of the arm. Contents: Physiological dose of saline. Dosage: 1 ml semi-annually at control visits. Packaging: 10 / 20ml vial with label applied, 1 ml drawn into clear, sterile disposable plastic syringe by blinded healthcare professionals immediately prior to injection. Packing: Eudract No. Subinvestigator contact information. ID number. Lot number. The text "Prolia® / Placebo". For NaCl, the labels are provided by the hospital pharmacy so that the blinded staff knows which patients need placebo. Batch no. on the given vial (which is also supplied by the hospital pharmacy), which is found in the ward, is registered on the label as well as in TMF. Placebo 2 (placebo for mimpara): Generic Name: Placebo (Placebo Centyl) Manufacturer: Jemopharm, Hasselvej 1, 4780 Stege Contents: Physiological dose of inactive minerals. 6.8x17 mm. Method of administration: Oral, green oval tablets with scored notch. Dosage: 1 pc daily. Packing: Eudract no. Subinvestigator contact information. ID number. Lot number. The text "Mimpara® / Placebo". Placebo for Mimpara: "Placebo Centyl" was originally produced for a similar experiment focusing on bone turnover, with Centyl as an intervention agent, where it has also been used as a placebo. Mimpara's color and size are comparable to placebo, as they are both green, measuring 6 x 10 mm and 7 x 17 mm respectively. Placebo has division notches and is thus not completely identical to the intervention preparation. We currently hold “Placebo Centyl” for a population of 50 patients. If more patients are included (up to 60 patients), additional Placebo Centyl will be purchased. Placebo has no effect on bone turnover. Placebo is packaged by the pharmacy staff in white, opaque Duma cans, and the counting and registration of experimental medication is done by blinded staff who are not investigators or sub-investigators, so the trial supervisor or delegated evaluating physician will be blinded. Trial participants are naturally not informed about the appearance of Mimpara / Placebo, which is why they will also be blinded in relation to the treatment. D vitamin. Supplied by D3 Pharmacy ApS, Bispensgade 22, 9000 Aalborg, Denmark as tablets of 25 micrograms each. Everyone is given a supplement of 50 micrograms daily unless they receive> 25 micrograms in advance. Vitamin D supplementation is given as vitamin D deficiency exacerbates primary hyperparathyroidism, may in itself lead to secondary (and

tertiary) hyperparathyroidism, and is included as standard in the treatment guidelines for patients with primary hyperparathyroidism.

Litterature review:

As previously mentioned, various pharmacological solutions to pHPT and their consequences have been tried over time. Particular focus has been on mitigating the effect on bone impact, and the genes due to the high s-calcium. Overall, the treatment can be divided into traditional antiresorptive drugs, as well as calcimimetics. Good effect on BMD, and lowering of S-Ca2 + and bone markers has been demonstrated for antiresorptive treatment with e.g. bisphosphonates15–17, estrogens and selective estrogen receptor modulators18–21 in previous studies. However, the effect on S-Ca2 + is temporary as one does not modulate parathyroid disease CaSR, so after a period one sees a rebound effect due to increasing amounts of PTH.

2.2 Resultats of non-clinical and clinical trials.

Cinacalcet:

Translation types

Text translation

Source text

2703 / 5000

Translation results

Administration of Cinacalcet is currently permitted in most countries for secondary HPT related to chronic kidney disease, as well as pHPT for parathyroid carcinoma. Cinacalcet has been shown in several studies to be effective in significantly lowering and normalizing s-Ca2 +, normalizing s-phosphate and, to a lesser extent, significantly lowering s-iPTH in patients with varying degrees of pHPT. 22–31 This effect has been shown in extension studies to the performed RCTs and retrospective studies to remain effective even over longer treatment periods (years) .28,32 The effect apparently does not depend on the etiology of the condition, and thus it has been shown that the same dose is required for effect in sporadic pHPT and MEN1 patients.22,33,34 Similarly, the drug has been found to be effective in lowering s-Ca2 + in patients with parathyroid carcinoma 31. The present studies suggest a slight increase in bone turnover assessed on bone formation and degradation markers, and BMD, u-calcium and creatinine remain unchanged during cinacalcet treatment. Thus, in cinacalcet treatment of primary hyperparathyroidism, additional treatment of these

endpoints will be required. The effect of cinacalcet on hypercalcemic symptoms or on hard endpoints such as fractures, kidney stones or cardiovascular complications has not been elucidated in RCT for patients with pHPT. The treatment triggers side effects relatively often (in about 25% of those treated), but these are by far the most mild - moderate, relatively rarely lead to discontinuation of treatment and increase with dose increase. A single study has systematically evaluated improved quality of life when taking Cinacalcet, suggesting this 25, but this needs to be confirmed by further studies. Faggiano et al found in a retrospective study of 23 patients with pHPT in cinacalcet treatment, of which 10 in combination with the bisphosphonate alendronate, that all treated patients received stabilized and controlled s-calcium, s-phosphate and u-calcium. Similarly, PTH decreased significantly. There was no difference in these parameters between the two groups. In patients with alendronate supplementation, BMD was assessed v. T-score increased by 9.6% in lumbar vertebrae and 3.9% in femur ($p < 0.01$). In patients treated with Cinacalcet alone, BMD remained unchanged.35. No other studies are known with cinacalcet in combination with antiresorptives currently with pHPT. In combination with alendronate, BMD increases, and combination therapy thus appears to be attractive to all patients with the combination of hypercalcaemia and osteoporosis based on pHPT. Additional studies with combination therapy of pHPT, including with Denosumab are requested in it 4th international workshop for medical treatment of asymptomatic pHPT.14

Denosumab:

Denosumab is a recombinant human IgG2 monoclonal antibody that inhibits RANKL. Decreased stimulation of the RANK receptor leads to inhibited osteoclast formation. The drug was first studied as a remedy in the treatment of osteoporosis in postmenopausal women. In the large 3rd phase FREEDOM trial, 7868 postmenopausal women with osteoporosis were randomized to treatment with subcutaneous 60 mg denosumab every 6 months, or placebo. After 3 year, denosumab had improved BMD (9.2% (95% CI, 8.2 to 10.1) in the lumbar spine, and 6.0% (95% CI, 5.2 to 6.7) in the hip) compared with placebo. Bone turnover markers were reduced (after 36 months CTx: 72% and P1NP: 76% according to placebo) and the incidence for vertebral, hip and nonvertebral fractures was significantly reduced (relative to placebo 68%, 40% and 20%, respectively). 36.37 In a substudy, vBMD was assessed by qCT increasing relative to the placebo group by 21.8%, 7.8%, and 5.9%, respectively, for the lumbar spine, hip, and femoral neck after 36 months (all $p < 0.0001$). Compared with placebo and baseline, a similarly significant increase in bone mineral content (BMC) was seen at the hip ($p < 0.0001$) and a significant BMC improvement in the trabecular, subcortical and cortical part of the bone ($p < 0.0001$) .38,39. Also the strength of the bone is shown improved, v. Finite element analysis (v. QCT), the bone strength is thus increased in the hip by 8.6% $p < 0.0001$ and in the vertebrae by 18.2% $p < 0.0001$ after 36 months compared to baseline .40 A follow-up extension study showed that long-term treatment (up to 8 years) maintains a still constructive effect in relation to BMD, and that the substance maintains bone markers for turnover at a low level. The incidence of vertebral and nonvertebral fractures also remained low with respect to placebo.41 This trend has been

confirmed by histological / histomorphological examination of bone biopsies in a total of 41 of the included patients who had been in treatment for up to 5 years at the time of biopsy.42 Another 2363 substudy of study participants examined a possible prophylactic effect of denosumab on aortic calcification, as well as on the incidence of cardiovascular events. Denosumab was not shown to have a significant effect over 3 years.43 A large number of studies have been performed confirming the above effects on aBMD, vBMD, bone markers (CTx and P1NP) and fractures in postmenopausal osteoporotic women.44–51 These effects have been shown in extension studies to continue to improve almost linearly over several years by continued treatment52–54. This is further substantiated in a recently published meta-analysis which, based on 4 of the above-mentioned RCTs with denosumab vs. Placebo finds a pooled effect with significant increase in BMD on lumbar spine (7.58%), hip (4.86%), and distal 1/3 of radius (2.92%) relative to placebo-treated (all, P <0.001). Patients treated with denosumab had a significant and very rapid decrease in CTx (-66.16%) and P1NP (-64.65%) compared to placebo-treated patients (both P <0.001). Adverse events were similar between the 2 groups (pooled odds ratio = 1.04, P = 0.625) .55 Another meta-analysis showed a slightly increased risk of infections with denosumab treatment according to placebo RR = 1.23 (1.00, 1.52), but no difference according to bisphosphonates RR = 1.13 (0.63, 2.03), and no other increased risks.56 Compared to bisphosphonates in patients with postmenopausal osteoporosis, denosumab has shown superiority in effect on both BMD and bone markers in a number of RCTs and open-label studies. 57–62, just as denosumab has been shown to be better at reducing cortical porosity in relation to alendronate, rated v qCT.63 Denosumab has also been studied in osteoporotic men64, in glucocorticoid treatment-associated osteoporosis60,65 and in bone loss associated with anti-hormone-based therapy in breast and prostate cancer, which is associated with increased fracture risk66–69. Here, too, denosumab has shown good efficacy in increasing BMD, lowering skeletal related events, lowering bone turnover measured by bone markers, and alleviating malignancy-associated hypercalcaemia. Denosumab has been shown in a single small open-label study to be able to increase BMD significantly in patients with secondary hyperparathyroidism. Denosumab has been shown to be effective in lowering s-calcium significantly. This effect can be exploited in hypercalcemic conditions .: In a small single-arm, open-label, proof-of-concept study in 15 patients with severe malignancy-induced treatment-refractory hypercalcaemia, Hu et al. Showed that denosumab in intensive therapy was able to lower s-calcium to endpoint (≤11.5 mg / dL) in 64% of patients within 10 days of first dose. Median uNTx / uCr decreased as expected. 70 This is supported by a number of case reports that primarily describe that Denosumab may be effective in treating refractory hypercalcemia in parathyroid carcinoma.71–75 However, the decreases in s-calcium have been shown to trigger a compensatory increase in s-iPTH in postmenopausal women osteoporosis76, which is why we in patients with pHPT predict a similar rebound effect of denosumab monotherapy.

Regarding side effects, the FREEDOM study using the same dose as in this trial did not find a significant difference in the incidence of adverse effects, mild to severe, between the 3900 who received denosumab and those who received placebo. Most commonly reported were

eczema and extremity pain. Likewise, there was no difference in the number who chose to stop treatment on the basis of side effects. The total amount of infection-related side effects was low and not significant, but numerically slightly higher than in the placebo group (especially cellulitis and eczema). The infections were heterogeneous in etiology and could not be related to the administration or duration of denosumab exposure.77 There was no increase in the risk of cancer, cardiovascular disease, delayed healing of fractures, or hypocalcaemia, and there were no cases of jaw osteonecrosis. In the extension study, 8 cases of jaw osteonecrosis and two cases of atypical fractures were added, which, considering the size of the study population, must be considered to be low.41 To sum up, one can briefly say that denosumab has shown superiority over bisphosphonates when it comes to increasing BMD, and lowering the risk of fractures in i.a. postmenopausal women with osteoporosis, as well as cancerous women and men in aromatase inhibitor and antiandrogen treatment, respectively. Likewise, it is more effective in lowering scalcium in malignant hypercalcaemia with solid bone metastases, and has also been shown to be effective in several case reports of refractory hypercalcaemia in patients with metastatic parathyroid cancer. Based on the known studies and meta-analyzes, Denosumab has been shown to be safe and without unacceptable side effects. There are no published studies in which denosumab has been tested in a controlled study in patients with primary hyperparathyroidism. Based on the above experiences from other patient groups, we believe that denosumab in combination with cinacalcet may be an effective medical treatment alternative to achieve good disease control in patients with pHPT, while improving BMD, lowering bone turnover and significantly reducing fracture risk in these patients.

Detrimental effects of pHPT:

Cardiovascular:

Increased cardiovascular mortality and morbidity are well described in classical primary hyperparathyroidism, just as it has been shown that this excess mortality decreases after parathyroidectomy.78–81 Increased s-iPTH has been shown in larger population studies to be associated with increased mortality and degree and risk of developing arteriosclerosis. 82.83 Limited and divergent data are available on cardiovascular outcome in asymptomatic pHPT. While some population studies suggest that there is no increased mortality associated with the condition84, others find a significantly increased cardiovascular morbidity and mortality.85. Several small studies have been performed on cardiovascular risk factors, such as hypertension, ventricular hypertrophy, coronary and valve calcification in asymptomatic pHPT. It is i.a. demonstrated that there is an association between high s-iPTH and aortic valve calcification, which has been shown to occur more frequently in patients with mild pHPT than in controls.86,87 The same authors conducted another small study in patients with mild pHPT, examining the effect of parathyroidectomy on signs of cardiovascular disease (carotis intima-media thickness, and stiffness, and aortic valve calcification), and found a modest positive effect on this 88. A small study has shown increased pulse wave velocity, indicative of incipient arteriosclerosis, in 24 patients with mild pHPT vs . matched control group.89 Conversely, a small study of 31 patients with mild pHPT compared to control group, by evaluation by MDCT could not demonstrate increased frequency of

coronary calcification.90 Overall, the studies suggest a subclinical impact, with increased vascular stiffness as the most consistent finding . Studies have been performed on the preventive effect of cinacalcet for cardiovascular morbidity and mortality secondary hyperparathyroidism due to chronic renal failure. These suggest a possible effect of Cinacalcet on the development of cardiovascular disease in secondary hyperparathyroidism in chronic renal disease91–94. There are no studies on the effect of Cinacalcet on cardiovascular disease or hard endpoints such as coronary calcification in pHPT. In this study, we want, although it is not the main focus of the study, to contribute to the evidence in this area. Many of the patients included in this trial are expected to belong to the group of patients with asymptomatic pHPT. Therefore, at CT we will examine the presence of coronary calcification, monitor blood pressure and record cardiovascular co-morbidity, and later examine whether there is a difference at baseline based on symptomatology and whether there is a difference in the development at the end of the study based on the treatment subgroups.

Kidneys and pancreas:
Asymptomatic hypercalciuria occurs in approx. 40% of patients with primary HPT.95. Hypercalciuria has been shown to be part of the background for stone formation in patients with symptomatic pHPT, as it contributes to the saturation of the urine with stone-forming calcium salts such as calcium phosphate and calcium oxalate.96 Cipriani et al found in a prospective study that kidney stones were found more often in symptomatic patients than in patients with asymptomatic pHPT (78% vs. 35.5%), this is a higher prevalence of kidney stones than retrospective studies have previously shown in patients with detected pHPT (eg 7% in asymptomatic patients 97 and in symptomatic patients 25.4% in 98 and 60% 96), and the study may be subject to selection bias. Nevertheless, the finding underscores the importance of monitoring for kidney stones in patients with asymptomatic pHPT. Brardi et al find in a small pilot study with ten patients with pHPT that cinacalcet in combination with calcium-normalized diet is able to significantly reduce the size and number of kidney stones shown by ultrasound and biochemistry.99 We will further elucidate this finding , by CT examine the presence of kidney stones and nephrocalcinosis, and evaluate prevalence in relation to symptoms, and possibly. effect of interventional pharmacies. The association between pHPT and acute and chronic pancreatitis is much debated. A systematic review reviewed the last 30 years of studies, and found a prevalence of pancreatitis of between 1.5% and 15% in a total of 10 studies on each of more than 50 trial participants.100 In this study, we want to examine patients with CT for prevalence of pancreatic calcifications and lesions, and see if necessary. development after 1 year of treatment. In this trial, in connection with qCT v. Baseline and after 1 year, we will co-scan the coronary artery, kidneys and pancreas to assess the incidence of calcifications, and progression thereof between treatment groups over time, including the difference in incidence in asymptomatic and symptomatic patients.

Literature search strategy:
Pubmed and embase were used. In pubmed the following MESH-terms were used ”Primary

Hyperparathyroidism*" combined with Mesh-synonyms for "Denosumab" and "Cinacalcet" combined and alone and furthermore using synonyms found in Embase. The following combinations of the terms Mesh- Denosumab and Cinacalcet, Denosumab and osteoporosis, Denosumab and hypercalcemia, PHPT and CT, -Cardiovascular Disease/Vascular Calcification/Coronary Artery Disease, - Chronic-/Calcific-/Acute Pancreatitis, and Nephrocalcinosis/Nephrolithiasis/Kidney Calculi were also used. In Embase the following combinations were used Primary Hyperparathyroidism combined with Cinacalcet and Denosumab, and Denosumab alone. All non-English literature was deselected. Original clinical trials with the following prioritation were selected RCT, prospectivc cohortstudies and retrospective cohortestudies. Chain-searching of reference lists of identified literature was performed in order to find any missed studies.

2.3 Summary of potential side effects, risks and potential benefits for the trial subjects.

Side effects and risks for the trial subjects:

Mimpara
Known side effects associated with treatment with Mimpara, see the manufacturer's package leaflet:

Very common: may affect more than 1 in 10 people
Nausea and vomiting. These side effects are usually quite mild and are not persistent.

Common: can affect up to 1 in 10 people
Dizziness, numbness or tingling sensation (paraesthesia), loss of appetite or decreased appetite, muscle pain (myalgia), fatigue (asthenia), rash, decreased testosterone levels, high potassium levels in the blood (hyperkalaemia), allergic reactions (hypersensitivity, or headache, cramps) low blood pressure, upper respiratory tract infection, difficulty breathing (dyspnoea), cough, indigestion (dyspepsia), diarrhea, abdominal pain - pain in the upper abdomen, constipation, muscle spasms, back pain, low calcium levels in the blood (hypocalcaemia). Not known: frequency cannot be estimated from the available data Urticaria, swelling of the face, lips, mouth, tongue or throat, which may cause difficulty swallowing or breathing (angioedema), abnormally fast or throbbing heartbeat, which may be associated with low levels of calcium in your blood (QT prolongation and ventricular arrhythmia secondary to hypocalcaemia).

Prolia:
Known side effects with Prolia:

Very common side effects (may affect more than 1 in 10 people):
Pain in bones, joints and / or muscles that can sometimes be severe, pain in the arms or legs (pain in the extremities).

Common side effects (may affect up to 1 in 10 people):
Painful urination, frequent urination, blood in the urine, involuntary urination (incontinence), upper respiratory tract infection, pain, tingling or numbness going down the legs (sciatica), blurred area of the lens of the eye (cataracts), constipation, stomach discomfort , rash, skin condition with itching, redness and / or dryness (eczema).

Uncommon side effects (may affect up to 1 in 100 people):
Fever, vomiting, abdominal pain or discomfort (diverticulitis), ear infection.

Rare side effects (may affect up to 1 in 1,000 people):
Allergic reactions (eg swelling of the face, lips, tongue, throat or other parts of the body, rash, itching or hives, wheezing or difficulty breathing). Patients receiving Prolia may develop skin infections in rare cases. As Prolia inhibits bone turnover, in very rare cases atypical fractures of bones and degradation of bone tissue in the jaw are seen. Radiation dose in connection with CT scan is associated with a well-known slightly increased risk of cancer. (see under the section on ethics). In addition, discomfort / inconvenience / time consumption in connection with blood sampling, clinical checks and completion of questionnaires. Benefits and possible benefits for the trial participant: The project provides new information on treatment options and prevalence of sequelae for patients with pHPT, as well as knowledge about the effect of denosumab on calcium metabolism and bone status in this group of patients. The project can thus contribute to the development of an effective medical treatment alternative for patents with pHPT. When studied with qCT, it will be possible to gain a more basic and detailed understanding of the impact on bone structure in patients with pHPT. We expect that the beneficial effects demonstrated in the studies reviewed above will benefit the trial participants in this study. Thus, we hope to be able to reduce / eliminate symptoms triggered by hypercalcaemia, increase BMD, and thus prevent and treat osteoporosis, bone fractures and possibly reduce the risk of developing kidney stones. By participating in the project, there is a theoretical possibility that the participants avoid progression in their condition in the long term, and thus they may avoid having to remove one or more parathyroid glands at a later operation. Risks associated with surgery are rare, but include complete or partial paralysis of the vocal cords, swallowing problems, permanent low calcium in the blood, bleeding, pain and infection.

2.4 Description and justification of dose, dosing, dosefrequency and treatment period.

Prolia:
Rationale for dose use and duration of treatment: The usual dose for the treatment of osteoporosis is 60 mg x 1 every 6 months. for the prevention of fractures. For the reduction of severe hypercalcaemia in malignant disease, doses as high as 120 mg at 8-day intervals may be used. For this study we will use: 60 mg, semi-annually for 1 year, svt a total of 2 injections. This dose is consistent with what has been used in trials in populations with other disorders.

Mimpara:

Rationale for dose use and duration of treatment: Treated for 1 year with 30 mg x 1 daily, for comparability with other clinical trials in the field, and to avoid the risk of hypokalemia.

D vitamin:
The vitamin D supplement is given to all participants, as a lack of vitamin D exacerbates primary hyperparathyroidism, can in itself lead to secondary (and tertiary) hyperparathyroidism, and is included as standard in the treatment guidelines for patients with primary hyperparathyroidism.

2.5 Description of the trial population:

The population will consist of patients who during the recruitment period (1 February 2017 - 1 April 2018) are diagnosed or monitored for primary hyperparathyroidism in the North Jutland Region, who have a reduced BMD with a T-score between -1.0 and -3.5, and who are not candidates for surgery or who waive an otherwise indicated surgery. (See further under the section on recruitment.)

3. Trial aims.

The aim of this randomized, double-blind, placebo-controlled study is to investigate whether treatment with Denosumab (ATC code: M05BX04) alone, or in combination with Cinacalcet (ATC code: H05BX01), may be an effective medical alternative for patients with low BMD pHPT who do not are surgery candidates. Particular emphasis is placed on the treatment of hypercalcaemia, the prevention of osteoporosis and the effect of the treatment on bone structure and circulation.

Fokusareas:

Primary aims:
Effect of intervention therapy on bone density, turnover and microstructure: To elucidate the effect of Denosumab, Cinacalcet or placebo with DXA of hip and spine, VFA of spine and q-CT of spine and distal 1/3 of radius v. Baseline and after 1 year in patients with pHPT, and decreased BMD.

Secondary aims:
-The effect of intervention treatment on biochemical disease control: Disease control and bone impact are continuously monitored by measuring s-ionized - and total Ca2 +, s- iPTH, s-phosphate, as well as bone markers in blood and urine over time (1 year). Occurrence and possible effect of treatment on other side effects of pHPT: In the course, s-amylase (pancreatic effect), s-creatinine (kidney effect), hs-crp, blood pressure, and ECG (cardiovascular effect) are measured. In connection with baseline and final qCT scan, the coronary artery (cardiovascular effect), kidneys and urinary tract (kidney effect) and the pancreas (pancreatic effect) are co-scanned. Thus, several, and ongoing, biochemical and imaging measurements of possible pancreatic, renal and cardiovascular effects are included. - Any reset of Ca-SR during prolonged treatment? To assess

whether prolonged suppression of s-Ca2 + and s-PTH can produce a reset of CaSR's sensitivity to S-Ca2 +, treatment is stopped after 1 year. 2 weeks after stopping treatment, s-Ca2 + and s-PTH are measured.

Trial hypotheses:

1. Treatment with a combination of denosumab and cinacalcet is more effective than monotherapy with denosumab and / or placebo in improving BMD and bone structure in patients with pHPT.
2. The CT part of a qCT can be used to detect coronary, pancreatic calcification, and nephrocalcinosis / lithiasis in patients with both symptomatic and asymptomatic pHPT.

3. The combination of denosumab and cinacalcet lowers Ca2 +, iPTH and bone markers –

4. Combination therapy with denosumab and cinacalcet may reset Ca-SR, thereby "curing" the disease.

4. Trial plan.

4.1 Primary and secondary endpoints.

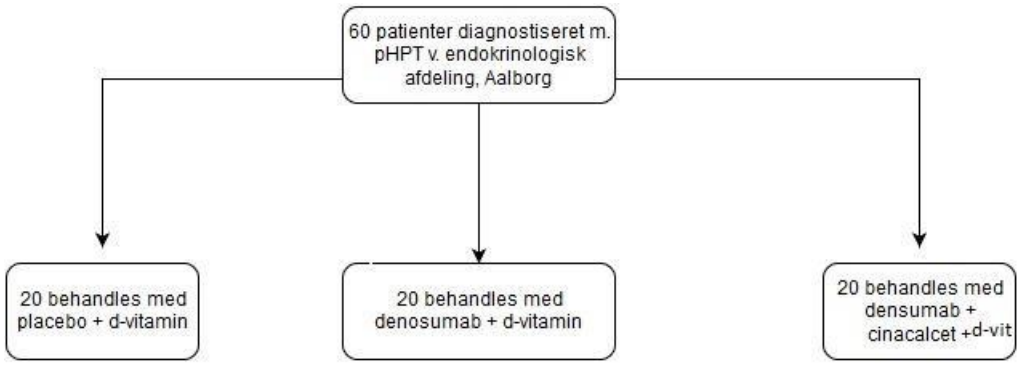
Primary endpoint:
-Percent change in BMD after 1 year of treatment from baseline and between intervention groups and placebo.

Secondary endpoints:
-Change in vBMD, BMC, cortical and trabecular thickness and ratio between them.
-Changes in s-calcium and s-PTH continuously and after 1 year of treatment.
-Percentage change in serum and urine bone markers
-Incidence / changes in the prevalence of coronary calcification assessed by agate tone score (and Ca2 + volume score).
- Development in possible nephrolithiasis / nephrocalcinosis assessed by CT - Development in possible pancreatic calcifications assessed by CT
-Possibly. reset of Ca-SR: measured by effect on p. Ca2 + and PTH at break (end) in treatment.
-A lateral DXA scan of the back (Vertebral Fracture Assessment (VFA) assesses the occurrence of osteoporotic collapses, including the relationship between anterior, middle and posterior compression.

4.2 Trial design

Et rand A randomized double-blind controlled single-center study.

Figure 1. Project flow diagram.



4.3 Measures for reducing or eliminating bias.

Bias is reduced by strict implementation of double-blindness and placebo control.

Medicine:

In this trial, denosumab is used in the form of Prolia, and cinacalcet in the form of Mimpara, both produced by Amgen and supplied by Sygehusapoteket, Aalborg University Hospital. Masking is planned so that both Prolia and placebo for Prolia (saline injection) will be administered without the patient or the person in charge of the trial seeing the syringe. The injections are thus planned to be performed by health personnel delegated by the person responsible for the experiment, who have thus been blinded. Tablets (Mimpara / Placebo) will be handed out in white plastic Duma cans, which will be delivered and packed by the hospital pharmacy, Aalborg, which will only be opened after the patient's return home. The patient will not be informed about the appearance of either placebo or Mimpara. Counting and registration of trial drugs will be performed by blinded healthcare professionals. The content of placebo will be resp. saline and mineral supplements without effect on bone turnover. During treatment visits in week 1, 1 treatment is submitted by the therapist (delegated). syringe s.c. containing either Prolia® or Placebo (saline). Duma cans are also handed out to each participant with tablets bearing the test name, subinvestigator's name, ID number, dose number, lot number and that the content is either "Mimpara®" or "Placebo". Sponsor guarantees that it is possible to blind at individual patient level without compromising the blindness of the other patients.

Randomization:

Randomization for intervention and placebo is performed at the hospital pharmacy, and is performed by number-based lottery. Each participant is randomized to one number, which is known by the participant and subinvestigator, but where group membership is unknown to both, thus both experimenters and participants are blinded. Information about group affiliation is stored on a randomization list at the hospital pharmacy and in locked rooms with the unblinded staff where the person responsible for the trial does not have access. The list can only be handed out to

experimenters after the experiment has ended. In the event of an urgent need for blinding of one or more participants, this will take place by the hospital pharmacy's 24-hour staff.

4.4 + 4.5 Trial treatment.

The trial will for the individual participant last 54 weeks from inclusion to final visit. 2 weeks before the final visit, the medication will be terminated, to measure any. continuously re-set by Ca-SR. There will be no inflow period. Extension of the study can be considered, framed for scientific and logistical reasons. In that case, this eventual case will then be notified in a separate protocol submission. Treatment in the trial consists of the intervention drugs denosumab (Prolia®), cinacalcet (Mimpara®) and vitamin D, as well as inactive placebo drugs. The dose of the intervention preparations is 60 mg denosumab / half-year, 30 mg cinacalcet / dgl. The placebo preparations may contain physiological doses of minerals or saline. Vitamin D contains 25 micrograms / tablet. Packing and labeling for dispensing placebos and intervention funds is handled by the hospital pharmacy, Aalborg University Hospital. The HDPE-Duma cans with intervention and placebo medicine are labeled with the experimental name, subinvestigator's name, randomization number, lot number and the text “Mimpara® / Placebo”. For Mimpara® and vitamin D as well as for placebo, oral dosing is taken with one tablet daily for morning administration. Prolia® and placebo injections are given at check-ups by healthcare professionals delegated by the investigator. The syringes will be either a Prolia syringe from the manufacturer (green / white) or a disposable plastic syringe with 1 ml of isotonic saline drawn up immediately before the test. The trial participant will not see the syringe administered by blinded delegated healthcare professionals. The treatment period for both intervention drugs and placebo is 1 year. Blood sample control is planned with attendance in the endocrinology department for the first time 1 week after entering the trial. Subsequent weeks 2, and 4 and thereafter check every 4 weeks. There will be a final interview and control at the termination of the experiment, where with the participant's consent, clarification of group placement and review of trial results at participant level will take place. 2 weeks after discontinuation of experimental medication, participants will be called in for an additional check-up in order to measure a possible increase in iPTH, and advice on further treatment.

4.6 Rules for stopping the entire trial and for the individual participant
Rules for stopping the entire trial:

In case of high incidence (more than 10 independent subjects), of predefined serious adverse events related to the pharmacology of the trial (serious adverse events); particularly severe and symptomatic hypocalcaemia, QT interval changes, aseptic bone necrosis, severe infections or atypical stress fractures. A similarly high incidence of unexpectedly serious side effects will of course lead to consideration of discontinuation of the trial, in which case the trial manager and sponsor will be discussed immediately.

Rules for stopping the experiment in the individual participant:
The following serious side effects have been assessed to be theoretical in relation to the treatment. When developing one or more of these, the experiment will be stopped for the individual. • Severe hypocalcaemia (ionized calcium <0.9 mmol / l; total calcium <1.45 mmol / l) Significant symptomatic / persistent hypocalcaemia (ionized calcium <1.10 mmol / l; total calcium <2.0 mmol /

l) • Severe infection • Aseptic bone necrosis • Atypical stress fractures • QT interval extension • Development of Angioedema The established exclusion criteria (see the section on this) with regard to medical treatment so far, and during the commencement of the trial for the individual participant, also apply during the course of the trial. Initiation of medical treatment, which is covered by the means on the exclusion criteria list, by a doctor treating at any given time, results in exclusion from the trial. Procedure for withdrawal of the experiment: If you wish to withdraw from the trial from the trial participant, a subsequent final visit is offered, with an agreement on further course, requisition of final tests and clarification of any. questions. In case of treatment need, a randomization number will be requested from the randomization list at the hospital pharmacy, which is staffed 24 hours a day, and affiliation with the intervention or placebo group will be revealed to the trial participant and subinvestigator. In the case of SAEs, the participant will be followed for development and clarification of causation in the event of withdrawal. If the reason for withdrawal is not of a therapeutic nature, the randomization number will be revealed to those responsible for the trial and will participate at the end of the trial in its entirety. Upon withdrawal, trial participants will be asked for a request for future updates regarding the trial's results, will receive the subinvestigator's contact information, and will be contacted during development in the trial that may have future treatment consequences for the resigned participant.

4.7 Procedures for trial drug accounting.

Dispensed trial drug is listed in a separate medicine folder of the unblinded staff to whom the person responsible for the trial does not have access. During monthly control visits, the trial participants are asked to bring all study-related drug packaging, including any remaining tablets since last visit. The trial participant is also asked about side effects of the trial drug and regularity in the intake of the preparations. Non-ingested tablets are indicated in the table whereby the total drug intake can be calculated. Furthermore, participants of the trial withdraw in the event of non-validity of participation, hereby defined as more than four days of consecutive failure to take the preparation, verified by the participant's information and brought medication. Similarly, absence from controls with subcutaneous drug administration will be grounds for withdrawal from the project.

4.8 CRF: Source data registered in the CRF are described in detail under the item ”Effectparameters”.

4.9 Randomisation code.

Code storage rules:
Linking between code and affiliation handed out to the subinvestigator and trial participant in either the intervention or placebo groups is stored exclusively on paper in the form of a randomization list with the unblinded staff, in rooms where trial managers do not have access, and at the hospital pharmacy which is staffed 24 hours a day. urgent need.

Code Breaking Rules:
Test code must be obtained by: • End of the experiment Development of serious side effects, where the trial participant withdraws from the trial, and where knowledge of affiliation in the intervention

or placebo group is revealed to the participant and subinvestigator with regard to categorization and statistical processing. Development of serious adverse reactions where an arbitrary treating physician treating the serious adverse reaction considers it necessary to obtain knowledge of affiliation in the intervention or placebo group and where the participant withdraws from the clinical trial.

5. Selection and exclusion of trial subjects.

5.1 Selection and screening of trial subjects.

Inclusion of up to 60 participants is sought according to the inclusion and exclusion criteria below. The participants will be patients who have been diagnosed with primary hyperparathyroidism at Endocrinology Departments in the North Jutland Region, by virtue of elevated sCa2 +, simultaneously elevated s-iPTH and exclusion of differential diagnoses. Participants will have a Tscore of between -1.0 and -3.5 for DXA scanning performed in connection with their assessment or monitoring. Participants will be patients who due to their comorbidity, own desire or other reasons do not meet the criteria for curative treatment in the form of surgery. These are thus patients with a disease requiring treatment, or a condition which in the long run will give rise to significant side effects, for which there is currently no satisfactory medical treatment offer. Pregnant or breastfeeding women are not involved. Participating women are asked if they are menstruating and are divided into fertile premenopausal women and infertile postmenopausal women. Fertile premenopausal women should submit to a negative pregnancy test prior to participation in the trial and use safe contraception throughout the study period as well as five times the blood half-life of the trial drug after the end of the trial. Safe contraception is defined as birth control pills, IUDs, depot injection of progestogen, subdermal implantation, hormonal vaginal ring and transdermal transdermal patches.

5.2 Inclusion criteria.

• Women and men over 18 years. • T-score rated by DXA between -1.0 and -3.5. • Patients from all over North Jutland examined and diagnosed with primary hyperparathyroidism at endocrinology outpatient clinics / wards, at hospitals in the North Jutland Region. This means persistent hypercalcaemia over at least two separate measurements with concomitantly elevated or inappropriately high normal s-iPTH and exclusion of differential diagnoses. (Regarding pregnancy - see exclusion criteria.)

5.3 Exclusion criteria.

Exclusion criteria summarized: • History of diseases that cause hypercalcaemia in addition to pHPT. • Patients treated with Prolia (denosumab) or Mimpara (Cinacalcet) • Patients previously treated with Mimpara • Moderate to severe hepatic impairment assessed by liver enzymes (ALT> 250u / l, GGT> 150u / l) and bilirubin> 30 micromol / l. • AMI or stroke within the last 3 months. • Known heart failure. • Risk factors for extended QTc interval. • Unhealed lesions from dental or

oral surgery. Primary bone diseases other than osteoporosis. • Patient with renal disease and renal insufficiency, including eGFR <30 mmol / L / 1.73m2, found at first biochemical requisition. • Patients on ongoing thiazide or lithium therapy. • Anamnesis with generalized seizures and epilepsy. • Active malignant disease. • Known allergy to intervention agents. • Pregnancy or breast-feeding. (positive pregnancy test v. inclusion) • Fertile women who do not want to use safe contraception. (By this is meant IUD or hormonal contraception (birth control pills, implants, transdermal transdermal patches, vaginal ring or transdermal injection). Conditions which, in the opinion of the subinvestigators and sponsors, make the participant unsuitable for carrying out the experiment, including a lack of understanding of the experiment or a lack of physical ability to participate.

Safety rules:

As cinacalcet is metabolised by the enzyme CYP3A4, patients will be orally and in writing advised against concomitant treatment with ketoconazole, macrolides, grapefruit juice, itraconazole, protease inhibitors, norfluoxetine, and voriconazole. Cinacalcet inhibits the enzyme CYP2D6 therefore patients should be advised against concomitant treatment with flecainide, mexiletine, propafenone, haloperidol, olanzapine, perphenazine, risperidone, thioridazine, zuclopenthixol, atenolol, metoprolol, propanolol, timolol, mipramin, mapitrile, amipriptin, amipriptin , codeine, dextromorphan, tramadol, fluoxetine, and paroxetine. Patients who are or during the trial period are being treated with the above preparations will be excluded from the trial in the development of side effects. From clinical experience, we know that these interactions are very rare. All patients who are potential participants will be discussed with regard to meeting the applicable inclusion and exclusion criteria between subinvestigator and sponsor.

5.4 Procedures for exiting the trial.

Upon the emergence of the safety parameters mentioned in section 8.3, the trial participant will withdraw from the trial immediately after its discovery. In the event of the occurrence of unexpected serious adverse reactions or conditions that may be suspected to be adverse events that will require hospitalization, the patient will, after discussion between the sponsor, sub-investigator and subject, withdraw from the trial. In the event of a serious illness requiring treatment in addition to those included in the trial (eg treatment-requiring cancer or AMI / stroke - which is also included in the exclusion criteria), the patient will be excluded from the trial. In other cases of diseases and side effects, any. Withdrawal depends on the overall assessment of the subinvestigators, sponsors / supervisors and trial participants. In case of withdrawal: Safety parameters that are objectively verified by biochemical values, radiological findings or clinically will be disclosed to the subinvestigator, who will then be responsible for telephone contact with the participant for the purpose of the trial, and requisition of final biochemical analysis package, which must be taken as soon as possible (when practical) possible. The subinvestigator will also record values obtained for research purposes. In the event of serious adverse events requiring hospitalization in the trial, the treatment will be handled by the treating physician at all times. Doctors who are delegated in the trial will be made aware in writing of incidents that lead to withdrawal from the project. Additional data will not be collected without renewed permission from the resigned participant after the end of

the trial. The participant is offered follow-up information regarding the results of the experiment and the outcome, if the participant is interested in this. Upon commencement of medical treatment by the physician in charge of treatment in primary, secondary and tertiary sectors, which at any time by the physician in charge of treatment is considered essential for the participants' further health and well-being, which are covered by exclusion criteria for the trial, the participant will withdraw from the trial. Resigned participants will only be reimbursed to the extent that recruitment is ongoing and the target number of participants of 45 has not yet been reached. In the case of 45 recruited participants, there will be no replacement of resigned participants. Further treatment and follow-up for retired trial participants will be handled by the relevant therapists in both the primary sector and the hospital sector.

6. Treatment of trial subjects.

6.1 Description of the treatment

For the individual participant, the trial will last 52 weeks from inclusion to the final visit + 2 weeks thereafter to the measurement of any. re-set by Ca-SR. There will be no inflow period. Extension of the study is considered, framed for scientific and logistical reasons. This possible case will then be notified in a separate protocol submission. Treatment in the trial consists of the intervention drugs denosumab (Prolia®), cinacalcet (Mimpara®) and vitamin D, as well as inactive placebo drugs. The dose of the intervention preparations is 60 mg denosumab / half year, 30 mg cinacalcet / dgl, and for vitamin D 50 micrograms / dgl. The placebo preparations may contain physiological doses of minerals without affecting bone and saline. Packaging for dispensing placebo and intervention funds is handled by the hospital pharmacy. Packaging with intervention and placebo medicine is labeled with the experimental name, subinvestigator's name, randomization number, lot number and the text “Mimpara® / Placebo”. For Mimpara® and vitamin D as well as for placebo, oral dosing is taken with one tablet daily for morning administration. Prolia® and placebo injections are given at check-ups by healthcare professionals delegated by the investigator. The syringes will be imprinted with “Prolia® / Placebo” and will either be a Prolia syringe from the manufacturer (Amgen) or a clear disposable plastic syringe with isotonic saline. The treatment period for both intervention drugs and placebo is 1 year. Blood test monitoring is planned with attendance in the endocrinology department 1st time 1 week after entering the trial. Subsequent weeks 2, and 4 and thereafter check every 4 weeks. There will be a final interview and control at the termination of the experiment, where with the participant's consent, clarification of group placement and review of trial results at participant level will take place. 2 weeks after the end of the trial and discontinuation of the trial drug, participants will be called in for an additional check-up in order to measure a possible increase in iPTH, and advice on further treatment.

6.2 Interventions on the trial subjects.

Biological material is requested in the form of blood samples, which require puncture of a vein on the forearm. No invasive procedures are performed in connection with imaging studies, and no requisition of biological material other than urine and blood samples is planned. When

administering prolia, the medicine is administered subcutaneously, which will take place once every six months, and is considered harmless from a procedural point of view.

6.3 Other drugs used by the trial subjects.

The trial participants' medication, in addition to the trial medication, is registered at each control visit in CRF. It is assessed here whether medication has been started up which may lead to exclusion from the trial, just as patients will be made aware of any risks of interactions. The responsibility for treatment other than that framed in the trial will be imposed on the relevant therapist who may start the treatment. Trial participants are allowed and instructed in, upon entering the trial, to bring information sheets regarding the trial treatment at all contacts with the health service.

6.4 Adherence.

Dispensed trial drug is listed in a separate medicine folder at the unblinded staff. During monthly control visits, the trial participants are asked to bring all study-related drug packaging, including any remaining tablets since last visit. This is also reviewed by the blind staff. The trial participant is also asked about side effects of the trial drug and regularity in the intake of the preparations. Non-ingested tablets are indicated in the table whereby the total drug intake can be calculated. Furthermore, participants of the trial withdraw in the event of non-validity of participation, hereby defined as more than four days of consecutive failure to take the preparation, verified by the participant's information and brought medication. Similarly, absence from controls with subcutaneous drug administration will be grounds for withdrawal from the project.

6.5 Check ups at the end of the trial and after the trial.

At the end of the trial for the individual participant, counseling and management of further treatment of the condition framed in the trial, (primary hyperparathyroidism) and complications thereof (osteoporosis, hypercalcemia) is handled by the Department of Endocrinology, Aalborg University Hospital. Other complications in the form of kidney stones, coronary stenoses, fractures, etc. will be handled under relevant auspices as well as the primary sector.

7. Evaluation of effect.

7.1 + 7.2 Effect parameters.

Radiological, biochemical and clinical markers are used to assess the treatment effect of both interventional drugs and placebo. Main radiological endpoint is percentage change in BMD and BMC in bone svt. distal radius, hip and lumbar spine after 52 weeks. For quantification of this, DXA scanning and VFA are used, with calculation of Z- and T-scores. T-score indicates, based on the BMD measured for the patient, the number of standard deviations above or below the mean for

a healthy 30-year-old of the same sex and ethnicity as the patient. Also assessed by qCT parameters such as vBMD, cortical and trabecular thickness, ratio between them, porosity and column collapse. This svt distal radius as well as the lumbar spine. These effect parameters have been chosen, as this allows information on bone architecture and strength to be obtained, which cannot be assessed by more traditional scanning methods. Thus, the application of qCT can contribute to a deeper understanding of the treatment's impact on bone structure, also for comparison with future similar projects. In connection with q-CT, the coronary area, kidneys and urinary tract, as well as the pancreas will also be scanned at baseline and after 1 year for examination of prevalence and development of coronary calcification, nephrocalcinosis, nephrolithiasis and for pancreatic calcification during the treatment period. This is quantified by Agatston scoring of coronary calcification, and radiological assessment of kidneys and pancreas. Thus, the incidence of, and a possible preventive effect on the development of side effects of primary hyperparathyroidism of treatment is assessed. The course duration of 1 year is decided for reasons of regularity between blood sampling and assessment of treatment effect for comparison with other studies and with regard to power calculation, where percentage change in BMD after 1 year is the starting point. Primary biochemical endpoints are absolute changes in values for blood sample packs termed “immediate analyzes” and “frost samples”. The content is listed below. For each session, control of "Immediate analyzes" in the form of ionized and total, alb corr. Calcium, s-phosphate, s-PTH, Hgb with EVF, acid-base status (T-CO2), infection parameters (CRP, leukocytes with diff.), electrolytes (Na + and K +), liver (ALT) and renal function (creatinine and eGFR) for the development of side effects and for understanding physiological changes due to the interventional drug. These are registered in the CRF. All other samples are frozen for later analysis called "Frost samples", these are only analyzed at the end of the experiment. The time horizons for the visits have been chosen on the basis of existing knowledge of the pharmacological agents. For the package "Bone markers", markers for bone breakdown and construction are used, for which the majority have been initiated at the Department of Clinical Biochemistry, Aalborg University Hospital, at the time of application. "Baseline" is divided into 2 visits, preliminary study and start visit as stated in the participant information.

Week	Base-line	1	2	4	8	12	16	20	24	28	32	36	40	44	48	52	54
Analysed immediately	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Samples for freezing	■					■			■			■			■		■
QCT	■															■	
DXA-scan	■															■	
VFA	■															■	
Clinical end epidemiological data	■															■	

Biochemical markers:
• "Immediate analyzes" Na +, K +, ALT, (hs) -CRP, differential count leukocytes, creatinine (eGFR), hemoglobin, hematocrit (EVF), albumin, T-CO2, Ca2 + ion-free, Ca2 + total (albumin-corrected), s-iPTH after drug-fasting, phosphate, at 1st visit further sedimentation reaction. • "Frost samples" Blood tests: urate, urea, GGT, bilirubin, amylase, lipid status, FSH, LH, free testosterone in men, bioavailable estradiol, 1,25 and 25-OH vitamin-D, magnesium, TSH, bone-specific alkaline phosphatase and methaneephrines. In addition, bone markers in the form of p-CTX, p-Trap5b, P1NP, bone-specific alkaline phosphatase, osteocalcin., FGF-23, and sclerostin. For urine frozen: Ucalcium, u-creatinine and u-phosphate on daily urine, which are collected at diagnosis, 24 weeks and 48 weeks. Time windows for controls: In general, control times are aimed at being as close as possible to what is stated schematically. For visits to control weeks 1, 2 and 4, the generally accepted time window will be +/- 3 days. For the inspections week 8 and onwards, the acceptable window will be +/- 10 days. Clinical data: Height, weight, BMI - Blood pressure and heart rate - Smoking status and alcohol consumption - Daily calcium intake - Dispositions, phpt, osteoporosis - Comorbidity - Medical treatment: Prednisolone, osteoporosis treatment, antihypertensives, hormonal treatment -EKG at 0.6, and 12 months. -MDI / HAMD depression score v. 0.6 and 12 months. Side effects are registered by form in CRF - see appendix. Requisition and analysis: Sampling, analysis and forwarding to the analytical department are handled at the Endocrinology Laboratory, Aalborg University Hospital. Samples are ordered by subinvestigator / delegates in the hospital's existing system ("Labka II"), where sampling from the printed PTB note of requisitioned samples is handed in on arrival at the Endocrinology Laboratory on given dates. A uniform sampling time is sought. Answers are returned to the subinvestigator in paper form for manual entry based on the code number in the data processing tool. Especially about selection and use of control group: In the experiment, a control group of up to 20 participants (1/3 of the participants) is used for comparison with the intervention groups m. Resp. Prolia and Prolia combined with Mimpara, in circumstances that would be quite similar to the traditional ones for patients with newly diagnosed primary hyperparathyroidism with a BMD within the stated framework, which is considered non-operable, as such patients today are basically simply observed on an ongoing basis. The control group is selected by lottery among all included patients. Addition or omission of surveys: If during the implementation of the study new biochemical parameters emerge which turn out to be better at characterizing the bone turnover than those mentioned above, the biochemical analyzes can be replaced / supplemented with these. Likewise, the subinvestigator reserves the right not to perform one or more of the above analyzes if it proves difficult to carry out for technical or economic reasons. Biochemical analyzes can only be omitted, replaced or changed after acceptance by the Science Ethics Committee.

8. Safety evaluation and handling of side effects and adverse events.

8.1 + 8.2 Known side effects, discomfort etc.
The disadvantages for the patients when participating in the trial are, in addition to inconvenience, time spent, and thus possibly lost earnings, primarily any side effects to the experimental drug. Known side effects of the intervention medication: Mimpara Known side effects associated with treatment with Mimpara, see package leaflet: Very common: may affect more than 1 in 10 people Nausea and vomiting. These side effects are usually quite mild and are not persistent. Common: can

hit up to 1 in 10 people Dizziness, numbness or tingling sensation (paraesthesia), loss of appetite or decreased appetite, muscle pain (myalgia), fatigue (asthenia), rash, decreased testosterone levels, high potassium levels in the blood (hyperkalaemia), allergic reactions (hypersensitivity, or headache, cramps) low blood pressure, upper respiratory tract infection, difficulty breathing (dyspnoea), cough, indigestion (dyspepsia), diarrhea, abdominal pain - pain in the upper abdomen, constipation, muscle spasms, back pain, low calcium levels in the blood (hypocalcaemia). Not known: frequency cannot be estimated from the available data Urticaria, swelling of the face, lips, mouth, tongue or throat, which may cause difficulty swallowing or breathing (angioedema), abnormally fast or throbbing heartbeat, which may be associated with low levels of calcium in your blood (QT prolongation and ventricular arrhythmia secondary to hypocalcaemia). Prolia: Known side effects with Prolia: Very common side effects (may affect more than 1 in 10 people): Pain in bones, joints and / or muscles that can sometimes be severe, pain in the arms or legs (pain in the extremities). Common side effects (may affect up to 1 in 10 people): Painful urination, frequent urination, blood in the urine, involuntary urination (incontinence), upper respiratory tract infection, pain, tingling or numbness going down the legs (sciatica), blurred area of the lens of the eye (cataracts), constipation, stomach discomfort , rash, skin condition with itching, redness and / or dryness (eczema). Uncommon side effects (may affect up to 1 in 100 people): Fever, vomiting, abdominal pain or discomfort (diverticulitis), ear infection. Rare side effects (may affect up to 1 in 1,000 people): Allergic reactions (eg swelling of the face, lips, tongue, throat or other parts of the body, rash, itching or hives, wheezing or difficulty breathing). Patients receiving Prolia may develop skin infections in rare cases. As Prolia inhibits bone turnover, in very rare cases atypical fractures of bones and degradation of bone tissue in the jaw are seen. Radiation dose in connection with CT scan and DXA scan is associated with a well-known slightly increased risk of cancer. (see under the section on ethics). In addition, discomfort / inconvenience / time consumption in connection with blood sampling, clinical checks and completion of questionnaires.

8.3 + 8.4 Safety parameters.

On the basis of proven, as well as theoretically possible, side effects for cinacalcet and denosumab, the trial is operated with the following safety parameters, which lead to withdrawal from the trial: As stated in section 4.6: "Rules for stopping the trial of the individual participant:" • Severe hypocalcaemia (ionized calcium <0.9 mmol / l; total calcium <1.45 mmol / l) Significant symptomatic and persistent hypocalcaemia (ionized calcium <1.10 mmol / l; total calcium <2.0 mmol / l). • Severe hospitalization infection • Aseptic bone necrosis • Atypical stress fractures • Development of extended QT interval • Development of severe renal insufficiency (GFR <30) • Development of Angioedema associated with ingestion of intervention drugs. Blood samples are taken to evaluate infection (CRP and leukocytes with differential count), acid-base status (T-CO2), kidney (Creatinine and eGFR), liver (ALT), electrolytes (Na + and K +), and calcium status (Ca2 + ion, Ca2 + total and albumin corr., S-phosphate and s-PTH) as well as Hemoglobin with hematocrit at each control visit for early biochemical detection of adverse events. At each follow-up visit, side effects, hospitalizations and treatments in the intervening period are asked. In relation to the drug denosumab, hypocalcaemia and its consequences are given priority in the selection of safety parameters. The development of hypocalcaemia, which has been shown to occur during treatment with denosumab, particularly in renal disease, and the theoretically possible but clinically very rare

side effects: increased tendency to infection (and therefore severe / recurrent infections), jaw osteonecrosis and stress fractures are defined as safety parameters withdrawal from the experiment, while relevant biochemical analyzes are requested. Similarly, in relation to the drug cinacalcet, electrolyte ranking and its consequences, especially within the spectrum of dyscalcaemia, are prioritized in the selection of safety parameters. The development of hypokalcaemia, as well as the very rare side effects of prolonged QT interval, and angioedema are defined as safety parameters and result in their onset withdrawal from the trial, while relevant biochemical analyzes are requested. The time frame for requisition is as soon as possible upon arrival at the relevant biochemistry department. The ECG is checked at the start of interventional medicine, after 6 and 12 months of treatment. Hypocalcaemia due to denosumab treatment has been reported to be particularly pronounced in renal disease, where treatment with calcium gluconate and vitamin d has been shown to be necessary. Therefore, patients with severe renal insufficiency (eGFR <30) will not be able to participate in the trial. In the case of new-onset hypocalcaemia, the condition will, if possible, be attempted with oral calcium supplementation. If s-ionized calcium can be brought to level, the experiment is continued. If this is not possible, the patient will be treated according to generally accepted guidelines (typically with calcium and vitamin D), and will be excluded from the trial as indicated. As cinacalcet is metabolised by the enzyme CYP3A4, patients will be orally and in writing advised against concomitant treatment with ketoconazole, macrolides, grapefruit juice, itraconazole, protease inhibitors, norfluoxetine, and voriconazole. Cinacalcet inhibits the enzyme CYP2D6 therefore patients should be advised against concomitant treatment with flecainide, mexiletine, propafenone, haloperidol, olanzapine, perphenazine, risperidone, thioridazine, zuclopenthixol, atenolol, metoprolol, propanolol, timolol, mipramin, mapitrile, amipriptin, amipriptin , codeine, dextromorphan, tramadol, fluoxetine, and paroxetine. Osteonecrosis of the jaw and possibly atypical fractures have been shown, as with bisphosphonates, to occur sporadically during treatment with denosumab. This is probably a class effect for all substances that effectively inhibit osteoclast function, and the incidence has been on a par with zoledronic acid and other bisphosphonates in reports and direct head-to-head studies. The incidence of jaw osteonecrosis overall for denosumab and bisphosphonates is by international consensus estimated at between 0.001% to 0.01%, at doses for the treatment of osteoporosis, which is marginally higher than the incidence in the general population (<0.001%). with denosumab has been associated with oncological doses (120mg / month) not used in this study. However, patients with non-healing wounds after tooth or jaw surgery will be excluded from the study. As RANKL is expressed in a number of tissues in addition to bone, including synovial, dendritic cells, T and B cells, there is a theoretical possibility that denosumab may be immunosuppressive. In the clinical trials reviewed, there has generally not been a statistically significant or clinically significant effect on the immune system. Long-term safety studies are still ongoing.102

8.5 Recording of adverse events/side effects.

Both serious and less serious adverse events are recorded in the patient record, and via CRF in the trial master file with information on whether the subinvestigator attributes association to trial medication. The symptoms that have arisen in this context, based on a medical assessment, will be compared with the product summaries for the drugs used. The events will be recorded indicating the affected organ system, as well as the severity of the event (mild, moderate, severe). Likewise, the

time relationship in relation to the intervention funds submitted will be registered. At any national level, any serious incident will be reported to the institution legally responsible for it at any time, whether it is pre-defined side effects or symptoms described by the trial participant, whether or not it is attributed to the trial drug by the subinvestigator / investigator. SAEs are thus reported immediately, and no later than within 24 hours by the subinvestigator to the sponsor who handles the further reporting, cf. 8.8. Admission will always be registered as SAE. Incidents / side effects are recorded in the patient record.

8.6 Measures to prevent and treat adverse effects.

Guideline in the event of a serious adverse event: Based on knowledge of existing adverse reactions to the interventional medicine, frequent checks on relevant biochemical variables are planned during the first 12 weeks of the trial with a view to early detection of inappropriate adverse reactions. In the event of the development of serious adverse events (both defined and undefined safety parameters), the trial participant will be instructed by the subinvestigator to discontinue the trial as soon as possible, potentially reversing several of the side effects, including hypocalcaemia, hyperkalaemia, hyperkalaemia. In the event of adverse reactions without reversibility of treatment discontinuation, treatment will follow current local and national guidelines and is expected to require hospitalization. In case of conditions requiring hospitalization, pt. of written information to the attending physician about conditions for withdrawal from the trial and immediate cessation of taking the trial drug. This experimental information is provided upon entry into the experiment. (See appendix "important information for doctors and other healthcare professionals"). A participant card will also be handed out with the trial name and information corresponding to that stated on the medicine labels, as well as the subinvestigator's name and contact information. At each control visit, participants will be asked about known symptoms and side effects of the intervention funds. (See Appendix Side Effect Questionnaire). In the event of side effects or other serious events, participants are informed to contact a physician, and then the person responsible for the trial. (see participant information). Upon withdrawal from the trial, the patient will be followed until the reason for withdrawal is noted and the biochemical termination analysis package is requested, as well as in the case of side effects, whether the outcome of the side effect will be fatal. In the event of the occurrence of non-exclusion-demanding side effects, where the investigator / delegate initiates any treatment, the participant will be followed up with closer checks for a period, depending on the given condition.

8.8 +8.9 Reporting of adverse events/side effects

The sponsor will immediately notify the National Board of Health if unexpected and serious suspected side effects occur during the trial. Sponsor shall ensure that all information about unexpected and serious suspected adverse reactions that are fatal or life-threatening is recorded and reported to The National Board of Health and the Ethics Committee of the North Jutland Region as soon as possible and no later than 7 days after the sponsor has become aware of such a suspected side effect. No later than 8 days after the report, the sponsor must notify the National Board of Health and the Science Ethics Committee North Jutland of all relevant information about the sponsor's and subinvestigator's follow-up on the report. All other unexpected and serious suspected

side effects must be reported to the National Board of Health and the Science Ethics Committee North Jutland no later than 15 days after the sponsor has become aware of these. Any report must be accompanied by comments on any consequences for the trial. All side effects and events are reported at the end of the trial in the final report to the National Board of Health and the Science Ethics Committee North Jutland. Subinvestigator must immediately report any serious incident to sponsor. The report must be followed up by a detailed written report, and in both the immediate report and the subsequent report, the subinvestigator must identify the subjects with a personal code number. The sub-investigator must also report to the sponsor incidents and / or abnormal analysis results that are stated in the trial protocol as being critical to the safety of the subjects. When reporting deaths, the subinvestigator must provide any additional information that the sponsor may request. The definitions are as follows, cf. the Executive Order for Clinical Trials: • incident: any adverse event in a patient or subject in a clinical trial after treatment with a medicinal product, without any connection between that treatment and the adverse event; • side effect: any harmful and unwanted reaction to a trial drug regardless of dose • unexpected side effect: a side effect whose nature or severity does not match the product information • serious incident or serious adverse reaction: an event or adverse event that, regardless of dose, results in death, is life-threatening, results in hospitalization or prolongation of hospital stay, results in significant or persistent disability or incapacity for work, or leads to a congenital anomaly or malformation. Reporting will be directed electronically to the SUSAR database (EudraVigilance Clinical Trial Module, E2B) using the e-form. A SUSAR is a Suspected Unexpected Serious Adverse Reaction: unexpected and serious suspected side effect. An adverse reaction is a SUSAR and must be reported when the following criteria are met: • severe, ie. result in death, be life-threatening, result in hospitalization or extension of hospital stay, • result in significant or permanent disability or incapacity for work or • lead to a congenital anomaly or malformation. • The side effect must be related to the test drug. That is, there is presumed to be a causal link between taking the drug and the side effect that occurred. Both the subinvestigator and the sponsor must assess causality. Sponsor must not disapprove of subinvestigator's assessment, and reports where sponsor does not agree with subinvestigator must be reported. • The side effect must be unexpected, ie a side effect whose nature or severity does not match the product information described in the SPC. As this is a blinded trial, the assessment of the adverse reaction must be carried out before blinding, but data must be blinded before reporting to the Danish Medicines Agency. As previously stated, sections on adverse reactions in the SPC for interventional medicine will be used as a reference in assessing the adverse reactions reported by trial participants, the causality assessment will always be based on a medical assessment.

8.10 Procedure for annual safety reports to the National Board of Health and the Science Ethics Committee.

Once a year during the entire trial period, a report is prepared listing all suspected serious adverse reactions that have occurred during the trial period. This is submitted to the Danish Medicines Agency and the Science Ethics Committee. To design this, a Form for reporting side effects is used, with an attached list. As mentioned above, SUSARS will also be reported immediately.

8.11 Procedure for reporting side effects/adverse events at the end of trial.

Upon termination of the trial, final reporting of adverse reactions to SST and VEK will be submitted within 90 days of the end of the trial, together with notification of the termination of the trial. The reporting of side effects here will be similar to the annual reports.

9. Statistics.

9.1 Statistical considerations:

When statistically calculating the data obtained, the use of different methods for the individual parts of the project is expected. No analysis is planned during the collection period, as in the controls, ongoing evaluation of biochemical signs of any serious side effects for the individual trial participant. All other data processing will take place exclusively after the end of the experiment. The following evaluations are planned to be carried out after the collection period: Intervention groups vs. Placebo and each other; All groups individually; development of effect goals; Symptomatic vs. Asymptomatic patients. Descriptive statistical methods are used for these populations; absolute values, mean, SD, SE, medians, quartiles (Q1, Q3), and range (Min, Max). For significance of difference between the groups, t-tests and wilcoxon signed rank according to indication can be used. Multiple regression analysis can be used in relation to confounder correction. Additional static methods will be considered in the course.

9.2 Power calculation.

The power calculation is based on the primary power measure BMD v. DXA scanning. With a risk of type 1 error of 5% and a risk of type 2 error of 20% (svt. A power of 80%) and an increase of 1% in BMD at DXA after 1 year with a Standard deviation on the BMD measurements of 1%, 15 participants must be used in each group. This calculation does not take into account dropouts, but since an SD of 1% is set high (in reality closer to 0.8), and in that case only 10 participants are needed in each group, it is expected to be sufficient in the calculated population size. Based on this, up to 60 trial participants will be included.

9.3 Applied significance level.

A statistical significance level of 5%, $P < 0,05$ will be used.

9.4 Criteria for end of trial.

Participants' completion of the final sample collection, DXA and QCT scanning as well as full attendance and for predetermined requisitions are considered completed trial participation if the medicine has been taken as agreed. The experiment ends at the notified date, or at the last participant's (60 in total) last final visit before that. Should the trial be extended, a separate request will be submitted.

9.5 Missing or unused data.

Missing data, including analysis errors, coagulation defective samples, will be considered missing values and not included in the analysis. In that case, the number of samples requested will be applied in each analysis (N). In the case of reference interval ultimate samples, which cannot be calculated in numerical value, these will also be categorized as missing values.

9.6 Deviations from the original statistical plan.
Deviations from the original statistical plan will be reported to the relevant authorities.

9.7 Trial subjects whose data will be used for the statistical analyses.
All subjects who have started treatment will be able to be included in the statistical analyzes.

10. Access to source data/source documents.

Upon request, full and unrestricted access to source data is provided to the local Science Ethics Committee, the National Board of Health, the Danish Data Protection Agency and the GCP unit, during prior consultation on ethical aspects regarding information attributable to specific individuals. Requests for access to source data for other countries' health authorities will depend on specific assessment and prior consultation with national Danish health authorities. Exchange of data with foreign and national research groups will only take place after concrete assessment and by anonymisation of sensitive personal information.

11. Quality controls.

The test will be performed according to the present test protocol, ICH-GCP guidelines and applicable legislation. The trial will be monitored at the GCP unit at Aalborg and Aarhus University Hospitals, Department of Clinical Medicine Aarhus University, Brendstrupgårdsvej 100, 8200 Aarhus N. Contact: Forskningens Hus in Aalborg, Søndre Skovvej 15, 9000 Aalborg, Main number: 9766 6266 The project follows general procedures for quality control and assurance.

12. Ethics

12.1 Ethical considerations.
The study will be conducted in accordance with the protocol and applicable legislation in the field. The experiment will be performed according to the Helsinki Declaration of Biomedical Research involving subjects. The primary ethical considerations in the trial relate to radiation hygiene in performing DXA and qCT scans, as well as the use of placebo in the presence of mild osteoporosis. Radiation hygiene: According to Chief Physician Jens Brøndum Frøkjær [1], from previous experiments at the Radiology Department, the radiation dose is known for the following studies: CT-KAG svt 0.5mSv, CT-upper abdomen 1 mSv qCT 3 vertebrae: 1.5 mSV Radiation dose at qCT of forearm is not known, but is stated in the international literature to <0.01mSv He estimates that the radiation dose at CT-KAG as well as CT-upper abdomen corresponds to the applied dose in the reported experiment with scanning of heart, pancreas and kidneys, with renal pelvis for calcifications. Total with a radiation dose of 1.5 mSV per study. qCT of the column loads as indicated with 1.5 mSv per scan. Each patient is thus expected to have a total radiation dose of approx. 6 mSv at the two scanning sessions. Corresponding to an overall increased risk of fatal

cancer of 0.03%, and thus an increase in overall risk from 25.0% to 25.03%. This is based on concrete calculations at the Department of Radiology, Aalborg University Hospital, and is consistent with findings in international publications. [2] Radiation dose at DXA, VFA and qCT of radius is so low that it is of negligible risk. The radiation dose corresponds to that measured in other Danish research projects, which have been approved by regional science ethics committees.

[1] Jens Brøndum Frøkjær, Overlæge, Radiologisk Afd., Aalborg Universitetshospital
[2] Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-raybased imaging techniques used in osteoporosis. Eur Radiol. 2010;20(11):2707-14

Placebo, osteoporosis and fractures.
Upon entering the trial, participants have verified cheesepene or mild osteoporosis from previous DXA scans performed for control or diagnostic purposes. Patients with moderate to severe osteoporosis (T-score <-3.5) will not be able to participate. Participants will all receive vitamin D supplements, and will also be randomized to treatment with either Prolia or placebo. Treatment with Vitamin D and Lime is standard treatment in people with osteopenia. Only people with mild osteoporosis (T-score between -2.5 and -3.5) can participate, and the treatment period is relatively short compared to the usual annual bone loss. Thus, we assess that the watchful waiting regime with vitamin D, which is standard in monitoring patients with primary hyperparathyroidism, and is actually what the patients in the placebo group are offered, can be justified in the listed setup. Should patients incur bone fractures during the treatment period, their continued participation in the trial will be reconsidered and discussed with the patient.

Benefits of participating in the trial.
The project adds new information about treatment options and prevalence of sequelae for patients with pHPT, as well as knowledge about the effect of denosumab on calcium metabolism and bone status in the studied group of patients. The project can thus contribute to the development of an effective medical treatment alternative for patents with pHPT. By examination with qCT, it will be possible to gain a new and more basic and detailed understanding of the impact of both the disease and the treatment on the bone structure in patients with pHPT, than what more traditional scanning methods can produce. We expect that the beneficial effects demonstrated in the studies reviewed in the background section will benefit the trial participants in this study. Thus, we hope to be able to reduce / eliminate symptoms triggered by hypercalcaemia, increase BMD, and thus prevent and treat osteoporosis, bone fractures and possibly reduce the risk of developing kidney stones. By participating in the project, there is a theoretical possibility that the participants avoid progression in their condition in the long term, and thus they may avoid having to surgically remove one or more parathyroid glands at a later operation. Risks associated with surgery are rare, but include complete or partial paralysis of the vocal cords, swallowing problems, permanent low calcium in the blood, bleeding, pain and infection.

12.2 Recruitment of trial participants and informed consent

Participants are recruited in several ways: via. day section and outpatient clinic in connection with control visits or assessment here, Department of Endocrinology, Aalborg University Hospital. Other possible candidates, all known in the department, are scheduled to be contacted by mail. Participants will be from the primary sector, or hospitals in the North Jutland Region referred for investigation and treatment of hypercalcaemia / pHPT. The assessment program typically includes blood tests, UL scan of the neck, DXA scan, and possibly scintigraphs and CT scans in search of focus. Upon detection of elevated PTH, as well as exclusion of differential diagnoses (including tertiary hyperparathyroidism, lithium treatment, pheochromocytoma, and familial hypercalcemic hypocalcury), the diagnosis of primary hyperparathyroidism is determined. Potential participants in their study were found to have a low BMD v. DXA scan with a T-score between -1.0 and -3.5. If the patient does not want or meet the criteria * for surgery, they are offered inclusion in the study. This will be offered by determining the diagnosis or by one of the patient's visits to the ward, or by positive feedback by letter. Materials regarding the study are submitted, with general information about the project's purpose, method and involvement of the patient (see cover letters and participant information). The pamphlets "Subjects' Rights" and "Before you decide" are also handed out. Interested parties will be invited to a visit per. letter and will receive oral information regarding the study. It will be possible for the person in question to contact the subinvestigator by telephone and via electronic and traditional text by agreeing to participate in an inclusion interview. (see appendix Participant information., Cover letters. Notice letters.) * Criteria for operation: Hypercalcemic symptoms • Nephrolithiasis, nephrocalcinosis or renal impairment • Age <50 years • Osteoporosis Serum calcium 10% above upper normal range (ionized plasma calcium> 1.45 mmol / l or albumin-corrected plasma calcium> 2.80 mmol / l)) Information call: Interested parties will be summoned for an interview with the possibility of a co-chair, which is mentioned in the summons letter, where the investigations and the course of study will be reviewed. The information interview takes place at Aalborg University Hospital in an undisturbed room (office) without switched on mobile phones / pagers, and is handled by Subinvestigator, Investigator or one of them delegated with the necessary professional knowledge. During the interview, the trial participant will be made aware of the possibility of a reflection period of up to 14 days prior to giving informed consent. Consent can be withdrawn at any time, and without justification. Only when the informed consent is available with the signatures of both the trial participant and the person responsible for the trial will the investigations be started. (consent form and package leaflets attached). Consent is registered on paper, and registered in the patient's electronic medical record. Package leaflets will be provided for the preparations Prolia® and Mimpara® and jointly side effects will be reviewed. In this context, information material is also handed out to health professionals. Predefined known serious side effects and the risk thereof will be informed, as well as for treatments, follow-up and convalescence at their onset. Participants will be instructed in the procedure for contacting the person responsible for the trial and the doctor at the onset of symptoms / side effects. The oral information interview is conducted by one of the medically trained experimenter, ie. Peter Vestergaard, Julius Simoni Leere, or one of the delegates responsible.

12.3 Privacy protection.

Forsøget er anmeldt til Datatilsynet og der er givet godkendelse for perioden 2016 – 2026.

The experiment has been notified to the Danish Data Protection Agency and approval has been given for the period 2016 - 2026. The information about the subjects is protected by the Personal Data Processing Act and the Health Act. External partners are not involved in EU or non-EU countries, and all biological material is stored and analyzed in Denmark. All trial contacts are registered in the EHR, where all information relevant to the patients' treatment is noted. The patients' other health information will be followed up via EHR, including documentation of adverse events, information on deaths and verification of information on existing diseases for analytical use. The requisition will be made with informed, written consent from participating patients. The trial will, after approval from the Science Ethics Committee, be notified to the Danish Medicines Agency.

12.4 Information from patient files.
Information on participants' health status, medication, and co-morbidity will be obtained upon enrollment in the study. This is to assess suitability in relation to inclusion, exclusion criteria and safety parameters. The information as well as demographic data can later be used for comparison between the subgroups in anonymised form. All information will be retrieved with your consent.

12.5 Trial subjects access to information on the project.

All participants receive contact information for the subinvestigator, who if they are interested will give them information regarding development in the project, publications, etc. An email list is created for this, which interested parties will provide contact information in connection with enrollment in the experiment. Possibly. info meeting about the result will be agreed at t later time.

13. Handling and acrhiving of data and biological material.

13.1 Datahandling.

Archiving of data will take place both manually and mechanically on a computer system. For each participant, paper responses received by inclusion interviews, questionnaires, X-ray scanning and biochemical analysis are saved. Responses from these requisitions are entered by the subinvestigator on personal computer with storage thereon and secondary as a backup on locally connected drive. There will be no storage on internet media. There will be password protection on computer and used storage media known only by subinvestigator and sponsor. Third party review and tampering will not be permitted without the agreement of the subinvestigator and sponsor. Data will be stored for the period notified to the Danish Data Protection Agency, and will be re-notified in the event of a request for an extension for permission to do so.

13.2 + 13.3 Biobank.

A biobank will be set up for the experiment. Collected biological material will be urine and blood taken in connection with planned control samples in the experiment. The biobank is used for

continuous freezing of urine and blood for analysis at the end of the experiment. Participants will be asked in advance to give their consent and including authorizing that the material may also be used for future research projects or further research in connection with the project, including the emergence of new markers for bone turnover. The storage will take place until 01 / 04-2026, when the material will be destroyed, or until the trial participant wants the material destroyed. Further permission for storage will be applied for if the material is to be used for projects after the specified time. Future research will require a new application and approval by Scientific Ethics Committee. There will be stored approx. 5-10 ml of blood, approx. 5-10 ml of urine pr. participant per. examination. There are no risks associated with taking samples to the biobank. The biobank will be notified to the Danish Data Protection Agency in accordance with current legislation.

14. Financing and insurance

14.1 Economy.

The trial is funded by existing research funds in the possession of sponsors from the North Jutland Region and Aalborg University. Fundraising is also planned, including funds from the Augustinús Foundation, AP Møller-Mærsk, Velux, Aase and Einar Danielsen's Foundation, as well as the Beckett Foundation. Funds allocated to the project will be deposited in a research account with the support of a sponsor. The initiative for the experiment was taken by the sponsor and subinvestigator without comment or amendments made by supporting parties. No agreement has been reached and will not be paid directly or indirectly for each participant. At the Department of Clinical Medicine, Aalborg University, the sponsor is in possession of research funds that are largely expected to be used for biochemical analyzes involved in the trial. The execution of radiological examinations is framed by the DRG tariff at the Department of Radiology, Aalborg University and will thus not directly or indirectly affect the operation of the experiment. Any excess support amount will be kept in the sponsor's custody under the Clinical Institute to finance future projects and analyzes, congress participants during which presentation of results can take place, or for future PhD projects. From Peter Vestergaard's professorship, we have the necessary funds for salaries, (1 part-time bioanalyst in 3 years - DKK 550,000; 1 PhD student for 3 years - DKK 1,548,000; 3 part-time supervisors - DKK 300,000), blood and urine samples (DKK 500,000), and scans (DKK 100,000 - ie a total of DKK 3,003,000). Funding for intervention medicine and placebo (Total approx.: DKK 535,000) is currently being sought from the above-mentioned funds. Should the fund applications fail, Sponsor, Peter Vestergaard has additional funds from an EU agency.er finansieret af eksisterende forskningsmidler i sponsors besiddelse fra Region Nordjylland og Aalborg Universitet.

14.2 Insurance

The trial is covered by insurance at Aalborg University and the patient insurance. Patients are covered by ordinary health insurance. Involved personnel are covered by resp. North Jutland Region and Aalborg University.

14.3 Payment of participants

No remuneration is given, but participants can receive transportation allowance according to current rules. The amount will reimburse specific petrol costs for car driving or train travel 2. class.

15. Publication

15.1 Publications

Results are planned to be published in international scientific journals. All results are given in anonymised form. Results will be published regardless of whether they are published in international journals or not, and whether their character is positive, negative or inconclusive. Subinvestigator will act as 1st author, sponsor and investigator as supervisor.

15.2 Reporting on trial end.

The trial will be completed at the last participant's last visit. This will be notified to the National Board of Health and the Science Ethics Committee North Jutland after 90 days from completion.

16. References:

1. Abood, A. & Vestergaard, P. Increasing incidence of primary hyperparathyroidism in Denmark. *Dan. Med. J.* **60,** 1–5 (2013).
2. Schaffalitzky De Muckadell, O. B., Haunsø, S. & Vilstrup, H. *Medicinsk Kompendium 17. Udgave*. (2009).
3. Christiansen, P. *et al.* Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. *Bone* **21,** 93–9 (1997).
4. Valdemarsson, S., Lindergard, B., Tibblin, S. & Bergenfelz, A. Increased biochemical markers of bone formation and resorption in primary hyperparathyroidism with special reference to patients with mild disease. *J Intern Med* **243,** 115–122 (1998).
5. Dempster, D. W. *et al.* Preserved three-dimensional cancellous bone structure in mild primary hyperparathyroidism. *Bone* **41,** 19–24 (2007).
6. Charopoulos, I. *et al.* Effect of primary hyperparathyroidism on volumetric bone mineral density and bone geometry assessed by peripheral quantitative computed tomography in postmenopausal women. *J. Clin. Endocrinol. Metab.* **91,** 1748–53 (2006).
7. Silva, B. C. *et al.* Trabecular bone score (TBS)--a novel method to evaluate bone microarchitectural texture in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **98,** 1963–70 (2013).
8. Stein, E. M. *et al.* Primary hyperparathyroidism is associated with abnormal cortical and trabecular microstructure and reduced bone stiffness in postmenopausal women. *J. Bone Miner. Res.* **28,** 1029–40 (2013).
9. Vu, T. D. T. *et al.* New insights into the effects of primary hyperparathyroidism on the cortical and trabecular compartments of bone. *Bone* **55,** 57–63 (2013).
10. Chen, Q. *et al.* Effects of an Excess and a Deficiency of Endogenous Parathyroid Hormone on Volumetric Bone Mineral Density and Bone Geometry Determined by Peripheral Quantitative Computed Tomography in Female Subjects. *J. Clin. Endocrinol. Metab.* **88,** 4655–4658 (2003).

11. Rubin, M. R. *et al.* The natural history of primary hyperparathyroidism with or without parathyroid surgery after 15 years. *J. Clin. Endocrinol. Metab.* **93,** 3462–3470 (2008).
12. Silverberg, S. J., Shane, E., Jacobs, T. P., Siris, E. & Bilezikian, J. P. A 10-year prospective study of primary hyperparathyroidism with or without parathyroid surgery. *N. Engl. J. Med.* **341,** 1249–1255 (1999).
13. Cipriani, C. *et al.* Prevalence of Kidney Stones and Vertebral Fractures in Primary Hyperparathyroidism Using Imaging Technology. *J. Clin. Endocrinol. Metab.* **100,** 1309–1315 (2015).
14. Bilezikian, J. P. *et al.* Guidelines for the Management of Asymptomatic Primary Hyperparathyroidism: Summary Statement from the Fourth International Workshop. *J. Clin. Endocrinol. Metab.* **99,** 3561–3569 (2014).
15. Chow, C. C. *et al.* Oral alendronate increases bone mineral density in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 581–7 (2003).
16. Khan, A. A. *et al.* Alendronate in Primary Hyperparathyroidism: A Double-Blind, Randomized, Placebo-Controlled Trial. *J. Clin. Endocrinol. Metab.* **89,** 3319–3325 (2004).
17. Rossini, M. *et al.* Effects of oral alendronate in elderly patients with osteoporosis and mild primary hyperparathyroidism. *J. Bone Miner. Res.* **16,** 113–119 (2001).
18. Grey, a B., Stapleton, J. P., Evans, M. C., Tatnell, M. a & Reid, I. R. Effect of hormone replacement therapy on bone mineral density in postmenopausal women with mild primary hyperparathyroidism. A randomized, controlled trial. *Ann. Intern. Med.* **125,** 360–368 (1996).
19. Orr-Walker, B. J. *et al.* Effects of hormone replacement therapy on bone mineral density in postmenopausal women with primary hyperparathyroidism: four-year follow-up and comparison with healthy postmenopausal women. *Arch. Intern. Med.* **160,** 2161–2166 (2000).
20. Rubin, M. R., Lee, K. H., McMahon, D. J. & Silverberg, S. J. Raloxifene lowers serum calcium and markers of bone turnover in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 1174–8 (2003).
21. Zanchetta, R. Raloxifene Reverses Bone Loss in Postmenopausal Women with Mild Asymptomatic Primary Hyperparathyroidism. **16,** 189–190 (2001).
22. Filopanti, M. *et al.* MEN1-related hyperparathyroidism: response to cinacalcet and its relationship with the calcium-sensing receptor gene variant Arg990Gly. *Eur. J. Endocrinol.* **167,** 157–64 (2012).
23. Khan, A. *et al.* Cinacalcet normalizes serum calcium in a double-blind randomized, placebocontrolled study in patients with primary hyperparathyroidism with contraindications to surgery. *Eur. J. Endocrinol.* **172,** 527–535 (2015).
24. Luque-Fernández, I., García-Martín, A. & Luque-Pazos, A. Experience with cinacalcet in primary hyperparathyroidism: results after 1 year of treatment. *Ther. Adv. Endocrinol. Metab.* **4,** 77–81 (2013).
25. Marcocci, C. *et al.* Cinacalcet reduces serum calcium concentrations in patients with intractable primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **94,** 2766–2772 (2009).

26. Norman, J., Lopez, J. & Politz, D. Cinacalcet (Sensipar) provides no measurable clinical benefits for patients with primary hyperparathyroidism and may accelerate bone loss with prolonged use. *Ann. Surg. Oncol.* **19,** 1466–71 (2012).
27. Peacock, M. *et al.* Cinacalcet hydrochloride maintains long-term normocalcemia in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **90,** 135–41 (2005).
28. Saponaro, F. *et al.* Cinacalcet in the management of primary hyperparathyroidism: post marketing experience of an Italian multicentre group. *Clin. Endocrinol. (Oxf).* **79,** 20–6 (2013).
29. Schwarz, P. *et al.* The PRIMARA study: a prospective, descriptive, observational study to review cinacalcet use in patients with primary hyperparathyroidism in clinical practice. *Eur. J. Endocrinol.* **171,** 727–35 (2014).
30. Shoback, D. M. *et al.* The calcimimetic cinacalcet normalizes serum calcium in subjects with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 5644–9 (2003).
31. Silverberg, S. J. *et al.* Cinacalcet hydrochloride reduces the serum calcium concentration in inoperable parathyroid carcinoma. *J. Clin. Endocrinol. Metab.* **92,** 3803–8 (2007).
32. Peacock, M. *et al.* Cinacalcet treatment of primary hyperparathyroidism: Biochemical and bone densitometric outcomes in a five-year study. *J. Clin. Endocrinol. Metab.* **94,** 4860–4867 (2009).
33. Giusti, F. *et al.* Cinacalcet therapy in patients affected by primary hyperparathyroidism associated to Multiple Endocrine Neoplasia Syndrome type 1 (MEN1). *Endocrine* (2015). doi:10.1007/s12020-015-0696-5
34. Moyes, V. J., Monson, J. P., Chew, S. L. & Akker, S. A. Clinical Use of Cinacalcet in MEN1 Hyperparathyroidism. *Int. J. Endocrinol.* **2010,** 906163 (2010).
35. Faggiano, a *et al.* Cinacalcet hydrochloride in combination with alendronate normalizes hypercalcemia and improves bone mineral density in patients with primary hyperparathyroidism. *Endocrine* **39,** 283–7 (2011).
36. Cummings, S. R. *et al.* Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N. Engl. J. Med.* **361,** 756–765 (2009).
37. Palacios, S. *et al.* Treatment with denosumab reduces secondary fracture risk in women with postmenopausal osteoporosis. *Climacteric* **7137,** 1–23 (2015).
38. Genant, H. K. *et al.* Improvements in hip trabecular, subcortical, and cortical density and mass in postmenopausal women with osteoporosis treated with denosumab. *Bone* **56,** 482–488 (2013).
39. McClung, M. R. *et al.* Denosumab densitometric changes assessed by quantitative computed tomography at the spine and hip in postmenopausal women with osteoporosis. *J. Clin. Densitom.* **16,** 250–256 (2013).
40. Keaveny, T. M. *et al.* Femoral and vertebral strength improvements in postmenopausal women with osteoporosis treated with denosumab. *J. Bone Miner. Res.* **29,** 158–165 (2014).
41. Papapoulos, S. *et al.* The effect of 8 or 5 years of denosumab treatment in postmenopausal women with osteoporosis: results from the FREEDOM Extension study. *Osteoporos. Int.* (2015). doi:10.1007/s00198-015-3234-7

42. Brown, J. P. *et al.* Effects of up to 5 Years of Denosumab Treatment on Bone Histology and Histomorphometry: The FREEDOM Study Extension. *J. Bone Miner. Res.* **29,** 2051–2056 (2014).
43. Samelson, E. J. *et al.* RANKL inhibition with denosumab does not influence 3-year progression of aortic calcification or incidence of adverse cardiovascular events in postmenopausal women with osteoporosis and high cardiovascular risk. *J. Bone Miner. Res.* **29,** 450–7 (2014).
44. Anastasilakis, A. D. *et al.* Comparative effect of zoledronic acid versus denosumab on serum sclerostin and dickkopf-1 levels of naive postmenopausal women with low bone mass: A randomized, head-to-head clinical trial. *J. Clin. Endocrinol. Metab.* **98,** 3206–3212 (2013).
45. Bekker, P. J. *et al.* A Single-Dose Placebo-Controlled Study of AMG 162, a Fully Human Monoclonal Antibody to RANKL, in Postmenopausal Women. *J. Bone Miner. Res.* **19,** 1059– 1066 (2004).
46. Bone, H. G. *et al.* Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. *J Clin Endocrinol Metab* **93,** 2149–2157 (2008).
47. Kumagai, Y., Hasunuma, T. & Padhi, D. A randomized, double-blind, placebo-controlled, single-dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of denosumab administered subcutaneously to postmenopausal Japanese women. *Bone* **49,** 1101–1107 (2011).
48. Lewiecki, E. M. *et al.* Two-year treatment with denosumab (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. *J. Bone Miner. Res.* **22,** 1832–1841 (2007).
49. McClung, M. R. Denosumab in postmenopausal women with low bone mineral density - Reply. *N. Engl. J. Med.* **354,** 2391 (2006).
50. Nakamura, T., Matsumoto, T., Sugimoto, T. & Shiraki, M. Dose-response study of denosumab on bone mineral density and bone turnover markers in Japanese postmenopausal women with osteoporosis. *Osteoporos. Int.* **23,** 1131–40 (2012).
51. Nakamura, T. *et al.* Clinical trials express: Fracture risk reduction with denosumab in Japanese postmenopausal women and men with osteoporosis: Denosumab Fracture Intervention Randomized Placebo Controlled Trial (DIRECT). *J. Clin. Endocrinol. Metab.* **99,** 2599–2607 (2014).
52. McClung, M. R. *et al.* Effect of denosumab on bone mineral density and biochemical markers of bone turnover: 8-year results of a phase 2 clinical trial. *Osteoporos. Int.* **24,** 227–235 (2013).
53. Miller, P. D. *et al.* Effect of denosumab on bone mineral density and biochemical markers of bone turnover: six-year results of a phase 2 clinical trial. *J. Clin. Endocrinol. Metab.* **96,** 394–402 (2011).
54. Sugimoto, T. *et al.* Three-year denosumab treatment in postmenopausal Japanese women and men with osteoporosis: results from a 1-year open-label extension of the Denosumab Fracture Intervention Randomized Placebo Controlled Trial (DIRECT). *Osteoporos Int* **26,** 765–774 (2015).

55. Gu, H.-F. *et al.* Efficacy and Safety of Denosumab in Postmenopausal Women With Osteoporosis. *Medicine (Baltimore).* **94,** e1674 (2015).
56. Zhou, Z. *et al.* Safety of denosumab in postmenopausal women with osteoporosis or low bone mineral density: A meta-analysis. *Int. J. Clin. Exp. Pathol.* **7,** 2113–2122 (2014).
57. Body, J.-J. *et al.* Effects of denosumab in patients with bone metastases with and without previous bisphosphonate exposure. *J. Bone Miner. Res.* **25,** 440–446 (2010).
58. Brown, J. P. *et al.* Denosumab significantly increases bone mineral density and reduces bone turnover compared with monthly oral ibandronate and risedronate in postmenopausal women who remained at higher risk for fracture despite previous suboptimal treatment with an oral bis. *Osteoporos. Int.* **25,** 1953–1961 (2014).
59. Kendler, D. L. *et al.* Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. *J. Bone Miner. Res.* **25,** 72–81 (2010).
60. Mok, C. C., Ho, L. Y. & Ma, K. M. Switching of oral bisphosphonates to denosumab in chronic glucocorticoid users: A 12-month randomized controlled trial. *Bone* **75,** 222–228 (2015).
61. Recknor, C. *et al.* Denosumab Compared With Ibandronate in Postmenopausal Women Previously Treated With Bisphosphonate Therapy. *Obstet. Gynecol.* **121,** 1291–1299 (2013).
62. Roux, C. *et al.* Denosumab compared with risedronate in postmenopausal women suboptimally adherent to alendronate therapy: Efficacy and safety results from a randomized open-label study. *Bone* **58,** 48–54 (2014).
63. Zebaze, R. M. *et al.* Differing effects of denosumab and alendronate on cortical and trabecular bone. *Bone* **59,** 173–179 (2014).
64. Langdahl, B. L. *et al.* A 24-Month Study Evaluating the Efficacy and Safety of Denosumab for the Treatment of Men With Low Bone Mineral Density: Results From the ADAMO Trial. *J. Clin. Endocrinol. Metab.* **100,** 1335–1342 (2015).
65. Petranova, T., Sheytanov, I., Monov, S., Nestorova, R. & Rashkov, R. Denosumab improves bone mineral density and microarchitecture and reduces bone pain in women with osteoporosis with and without glucocorticoid treatment. *Biotechnol. Biotechnol. Equip.* **28,** 1127–1137 (2014).
66. Ellis, G. K. *et al.* Randomized trial of denosumab in patients receiving adjuvant aromatase inhibitors for nonmetastatic breast cancer. *J. Clin. Oncol.* **26,** 4875–4882 (2008).
67. Martin, M. *et al.* Bone-related complications and quality of life in advanced breast cancer: Results from a randomized phase III trial of denosumab versus zoledronic acid. *Clin. Cancer Res.* **18,** 4841–4849 (2012).
68. Gnant, M. *et al.* Adjuvant denosumab in breast cancer (ABCSG-18): a multicentre , randomised , double-blind , placebo- controlled trial. *Lancet* **6736,** 1–11 (2015).
69. Planas Morin, J. *et al.* Bone mass behavior after 1 year of different treatment strategies in prostate cancer patients subjected to androgen deprivation therapy. *Rheumatol. Int.* **34,** 1419–25 (2014).

70. Hu, M. I. *et al.* Denosumab for patients with persistent or relapsed hypercalcemia of malignancy despite recent bisphosphonate treatment. *J. Natl. Cancer Inst.* **105,** 1417–1420 (2013).
71. Bech, A. & de Boer, H. Denosumab for tumor-induced hypercalcemia complicated by renal failure. *Ann. Intern. Med.* **156,** 906–7 (2012).
72. Karuppiah, D. *et al.* Refractory hypercalcaemia secondary to parathyroid carcinoma: Response to high-dose denosumab. *Eur. J. Endocrinol.* **171,** K1–K5 (2014).
73. Nadarasa, K. *et al.* Denosumab for management of refractory hypercalcaemia in recurrent parathyroid carcinoma. *Eur. J. Endocrinol.* **171,** L7–8 (2014).
74. Tong, C. V, Hussein, Z., Noor, N. M., Mohamad, M. & Ng, W. F. Use of denosumab in parathyroid carcinoma with refractory hypercalcemia. *QJM* **108,** 49–50 (2015).
75. Vellanki, P., Lange, K., Elaraj, D., Kopp, P. a & El Muayed, M. Denosumab for management of parathyroid carcinoma-mediated hypercalcemia. *J. Clin. Endocrinol. Metab.* **99,** 387–90 (2014).
76. Makras, P. *et al.* Parathyroid hormone changes following denosumab treatment in postmenopausal osteoporosis. *Clin. Endocrinol. (Oxf).* **79,** 499–503 (2013).
77. Watts, N. B. *et al.* Infections in postmenopausal women with osteoporosis treated with denosumab or placebo: Coincidence or causal association? *Osteoporos. Int.* **23,** 327–337 (2012).
78. Agarwal, G. *et al.* Cardiovascular dysfunction in symptomatic primary hyperparathyroidism and its reversal after curative parathyroidectomy: results of a prospective case control study. *Surgery* **154,** 1394–403; discussion 1403–4 (2013).
79. Hedbäck, G. M. & Odén, A. S. Cardiovascular disease, hypertension and renal function in primary hyperparathyroidism. *J. Intern. Med.* **251,** 476–483 (2002).
80. Osto, E. *et al.* Coronary microvascular dysfunction induced by primary hyperparathyroidism is restored after parathyroidectomy. *Circulation* **126,** 1031–9 (2012).
81. Vestergaard, P. *et al.* Cardiovascular events before and after surgery for primary hyperparathyroidism. *World J. Surg.* **27,** 216–222 (2003).
82. Hagström, E. *et al.* Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation* **119,** 2765–2771 (2009).
83. Hagström, E. *et al.* Plasma-parathyroid hormone is associated with subclinical and clinical atherosclerotic disease in 2 community-based cohorts. *Arterioscler. Thromb. Vasc. Biol.* **34,** 1567–73 (2014).
84. Wermers, R. a *et al.* Survival after the diagnosis of hyperparathyroidism: a population-based study. *Am. J. Med.* **104,** 115–22 (1998).
85. Yu, N. *et al.* Increased mortality and morbidity in mild primary hyperparathyroid patients. The Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin. Endocrinol. (Oxf).* **73,** 30–4 (2010).
86. Anderson, J. L. *et al.* Parathyroid hormone, vitamin D, renal dysfunction, and cardiovascular disease: Dependent or independent risk factors? *Am. Heart J.* **162,** 331–339.e2 (2011).
87. Iwata, S. *et al.* Aortic valve calcification in mild primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **97,** 132–7 (2012).

88. Walker, M. *et al.* Effect of Parathyroidectomy on Subclinical Cardiovascular Disease in Mild Primary Hyperparathyroidism. *Eur. J. Endocrinol.* **167,** 277–285 (2012).
89. Schillaci, G. *et al.* Large-artery stiffness: A reversible marker of cardiovascular risk in primary hyperparathyroidism. *Atherosclerosis* **218,** 96–101 (2011).
90. Kepez, A. *et al.* Evaluation of subclinical coronary atherosclerosis in mild asymptomatic primary hyperparathyroidism patients. *Int. J. Cardiovasc. Imaging* **25,** 187–93 (2009).
91. Evolve, T. & Investigators, T. Effect of Cinacalcet on Cardiovascular Disease in Patients Undergoing Dialysis. *N. Engl. J. Med.* **367,** 2482–2494 (2012).
92. Parfrey, P. S. *et al.* The Effects of Cinacalcet in Older and Younger Patients on Hemodialysis: The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial. *Clin. J. Am. Soc. Nephrol.* **10,** 791–799 (2015).
93. Raggi, P. *et al.* The ADVANCE study: A randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. *Nephrol. Dial. Transplant.* **26,** 1327–1339 (2011).
94. Urena-Torres, P. A. *et al.* Protocol adherence and the progression of cardiovascular calcification in the ADVANCE study. *Nephrol. Dial. Transplant.* **28,** 146–152 (2013).
95. Wüthrich, R. P., Martin, D. & Bilezikian, J. P. The role of calcimimetics in the treatment of hyperparathyroidism. *Eur. J. Clin. Invest.* **37,** 915–22 (2007).
96. Odvina, C. V *et al.* Biochemical characterization of primary hyperparathyroidism with and without kidney stones. *Urol. Res.* **35,** 123–8 (2007).
97. Suh, J. M., Cronan, J. J. & Monchik, J. M. Primary hyperparathyroidism: is there an increased prevalence of renal stone disease? *AJR. Am. J. Roentgenol.* **191,** 908–11 (2008).
98. Linde, J. S., Waldhauer, E., Rolighed, L., Mosekilde, L. & Vestergaard, P. Renal stones and calcifications in patients with primary hyperparathyroidism: Associations with biochemical variables. *Osteoporos. Int.* **23,** S93 (2012).
99. Brardi, S., Cevenini, G., Verdacchi, T., Romano, G. & Ponchietti, R. Use of cinacalcet in nephrolithiasis associated with normocalcemic or hypercalcemic primary hyperparathyroidism: results of a prospective randomized pilot study. *Arch. Ital. Urol. Androl.* **87,** 66–71 (2015).
100. X. Bai, H., Giefer, M., Patel, M., Orabi, A. I. & Husain, S. Z. The Association of Primary Hyperparathyroidism With Pancreatitis. *J Clin Gastroenterol* **46,** 656–661 (2012).
101. Khan, A. a *et al.* Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. *J. Bone Miner. Res.* **30,** 3–23 (2015).
102. Ferrari-Lacraz, S. & Ferrari, S. Do RANKL inhibitors (denosumab) affect inflammation and immunity? *Osteoporos. Int.* **22,** 435–446 (2011).

List of abbreviations:

BMC: Bone Mineral Content
BMD: Bone Mineral Density
BSAP: Bone Specific Alkaline Phosphatase (formations markør) **CaSR:**
Calcium sensing receptor.
CTx: Carboxy-terminal collagen crosslinks (resorption marker)
DXA: Dual-energy X-ray absorptiometry
FGF-23: Fibroblast growth factor 23
HRqCT: High Resolution Quantitative Computed Tomography
MDCT: Multidetector Computed Tomography
MEN1: Multiple Endocrine Neoplasia type 1
Osteocalcin: Formationsmarker
pHPT: Primary Hyperparathyroidism
P1NP: Serum type 1 procollagen (N-terminal)
qCT: Quantitative Computed Tomography
RANKL: Receptor activator of nuclear factor kappa-B ligand
Sclerostin: Inhibition of bone formation.
sHPT: Secondary Hyperparathyriodism
s-iPTH: serum – intakt parathyroidhormon
uNTx: Urinary collagen type 1 cross-linked N-telopeptid
VF: Vertebral Fractures
VFA: Vertebral Fracture Assessment
T-score: Deviation from BMD of a 30 year old subject of the same gender

Final statistical analysis plan

(from the final protocol)

4. Study Plan:

4.1 Primary and Secondary Endpoints.

Primary endpoint:

-Percentage change in BMD (by DXA) after one year of treatment from baseline.

Secondary endpoints:

-Changes in volumetric BMD, BMC, cortical and trabecular width as well as ratio between these.
-Changes in s-calcium and s-PTH at each visit and after one year of treatment.
-Percentage change in serum- and urine- bone turnover markers
- Prevalence/changes in coronary calcifications by Agatston-score (and Ca2+-volume score).
-Development in nephrolithiasis/nephrocalcinose by CT
-Development in pancreatic calcifications by CT
-Possible reset of the Calcium sensing receptor: measured by effect on serum-Ca^{2+} and PTH after the termination of the study-treatment.
-Prevalence of osteoporotic vertebral frectures by Vertebral Fracture Assessment (VFA), including the relationship between anterior, mid- and posterior compressions.

7. Impact Assessment

7.1 + 7.2. Efficacy Endpoints

For the evaluation of efficacy of the study-treatment (active as well as placebo) radiological, biochemical and clinical endpoints will be investigated. The most important radiological endpoints are percentage change in BMD and BMC in at the distal radius, hip and lumbar column at 52 weeks. To quantify this, DXA-scans with VFA, including calculations of Z- and T-scores will be used. The T-score indicate, based on the individual BMD, the number of standard-deviations above or below the mean for a healthy person of the same sex and ethnicity at 30 years of age. Similarly QCT-parameters such as vBMD, cortical and trabecular width (and ratio between these), porosity, and vertebral fractures will be investigated (at the distal radius and lumbar column). These endpoints (QCT) have been chosen because they allow for evaluation of bonearchitecture and strength, which cannot be investigated by more traditional scan-methods (DXA). Hence, the use of QCT can contribute to the achievement of a deeper understanding of the impact of the treatment on bone-structure.

In relation to the QCT-scans at baseline and after one year, images of the coronary arteries, kidneys, and the pancreas will be obtained for evaluation of prevalence and development of coronary calcifications, nephrocalcinosis, nephrolithiasis and pancreatic calcifications in the treatment-period. These endpoints will be quantified by Agatston-scoring of coronary calcifications, and radiological assessment of the kidneys and the pancreas. This allows for assessment of a potential preventive effects of the treatment on the development of known sequlae/targets of primary hyperparathyroidism.

The duration of the intervention-period (1 year) has been chosen to standardize the regularity of bloodsampling and powercalculation (based on the percentage changes in BMD over one year) and to allow for comparison of treatment effect with previous trials.

The biochemical endpoints are measured by absolute change in blood-test-parameters for the following collections of tests: “immediate analyses” and “frozen samples”. The contents are listed below.

At each session control of “immediate analyses” are planned.These include ionized calcium, total albumin adjusted calcium, s-phosphate, s-PTH, hemoglobin and erythrocyte volume fraction, acid-base status (TCO_2), infection-parameters (CRP, WBC w. diff.), electrolytes (Na^+ and K^+), liver (ALAT), and kidneyfunction(creatinine and eGFR). These tests are measured at each visit for safety purposes and to gain a better understanding of the physiological changes induced by the study-treatment. These parameters are registered in the individual case report forms.

All additional blood-samples are frozen for later analysis after the end of the study. The timeframe for the included control-visits has been chosen based on the available knowledge on the effects of the studymedicine.
For analysis of “Bone-turnover markers”, markers of bone resorption and –formation will be used of which the majority are available at the Department of Clinical Biochemistry, Aalborg University Hospital, at the time of application.

”Baseline” is divided in 2 separate visits, preliminary examination and start-visit.

Week	Base-line	1	2	4	8	12	16	20	24	28	32	36	40	44	48	52	54
”Immediate analyses”	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
”Frozen Samples”	■					■			■			■			■		■
QCT	■															■	
DXA-scanning	■															■	
VFA	■															■	
Clinical and epidemiological data	■															■	

Biochemical markers:
• ”Immediate analyses”
Na^+, K^+, ALAT, (hs)-CRP, WBC w. diff, creatinine(eGFR), hemoglobin, erythrocyte volume fraction, albumin, T-CO_2, Ionized Calcium, total albumin adjusted calcium, s-PTH after pausing study medicine, phosphate, and at baseline, additionally erythrocyte sedimentation rate.

• ”Frozen Samples”
Blood-tests: urate, carbamid, GGT, bilirubin, amylase, lipid-status, FSH, LH, free testosterone, estradiol, 1,25 and 25-OH-vitamin-D, magnesium, TSH, bone specific alkaline phosphatase and metanephrines. In addition to these, bone turnover markers in the form of p-CTX, p-Trap5b, P1NP, bone-specific alkaline

phosphatase, osteocalcin ,FGF-23, and sclerostin. Furthermore (24hr) urine samples are frozen: U-calcium, u-creatinine and u-phosphate collected at baseline, 24 weeks and 48 weeks.

Timeframe for control-visits:
Generally control-visits are planned carried out as accurately as possible according to the plan above. For visits at weeks 1, 2 and 4 from start, the acceptable timeframe will be +/-3 days. For the remaining visits (weeks 8 and onward) the acceptable timeframe will be +/-10 days.

Clinical data:
-Height, weight, BMI
-Bloodpressure and pulse
-Smoking status and alcohol consumption
-Daily calcium-intake
-Familial dispositions, PHPT, osteoporosis
-Co-morbidities
-Pharmacological history: Prednisolone, osteoporosis treatment, anti-hypertensives, hormonal treatment - EKCG at 0,6, and12 mths.
-MDI/HAMD depression-scores at 0,6 og 12 mths.
- Adverse events are registered by questionnaire in the CRF.

9. Statistics:

9.1 Statistical considerations:

The collected data from the different parts of the project is planned to be analyzed by different methods. No analyses of data is planned to be carried out prior to the end of the study, except for evaluation of potential reported and biochemical adverse events on an individual level. All other data management will take place after the end of data collection.

The following evaluations are planned to take place after the end of the study:
Comparisons of the variables listed above, between the three groups (by study-treatment); Development in endpoints within all groups.
Symptomatic vs. Asymptomatic patients (Symptoms of PHPT).

For these comparisons descriptive statistical methods will be applied; absolute values, means, standard deviations and standard errors, medians, quartiles(Q1, Q3), and range (Min, Max).
For testing of significance of differences between groups, t-tests and wilcoxon signed rank tests (parametric and non-parametric tests) per indication can be applied. Multiple regression-analysis can be applied in relation to investigation of confounder-correction. Further statistical methods will be considered before the analysis.

9.2 Power calculation.

The power-calculation is based on the primary endpoint BMD by DXA-scan.
With a risk of type 1 errors of 5% and a risk of type 2 errors of 20% (a power of 80%) and an expected increase of 1% in BMD by DXA after 1 year with a standard-deviation for the BMD-measurements at 1%, 15 are needed in each group.
This calculation does not account for dropouts, but since a SD of 1% is a high estimate (in reality closer to 0.8%, in which case only 10 participants in each group are needed), the calculated population-size is expected to be adequate.
Based on this, up to 60 participants will be sought to be included.

9.3 Significance level applied.

P-values <0.05 are considered significant

9.5 Missing data.

Missing data, including analysis errors and faulty blood-tests, will not be included in the analyses. In case of missing data, the number of analyzed results (n) will be reported. Reference interval-ultimate results, that cannot be converted to a numerical value similarly will be categorized as missing data. All participants that have initiated study-treatment will be included in the statistical analyses.