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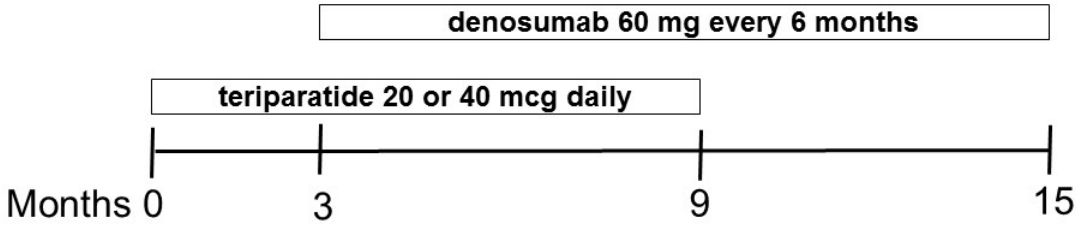
Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Tsai J N, Lee H, David N L, et al. Combination denosumab and high dose teriparatide for postmenopausal osteoporosis (DATA-HD): a randomised, controlled phase 4 trial. *Lancet Diabetes Endocrinol* 2019; published online August 22. http://dx.doi.org/10.1016/S2213-8587(19)30255-4.

Appendix.

Supplementary Figure 1: DATA-HD study design.



Detailed Protocol: Denosumab and High-dose Teriparatide Administration Study (DATA 2)

I. Background and Significance

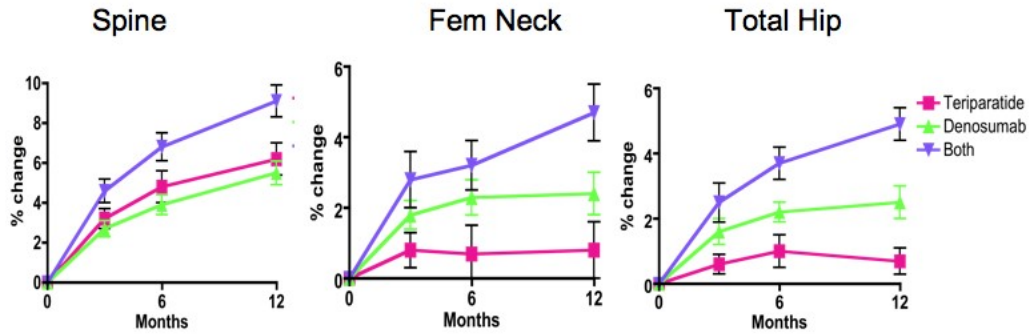
As our population ages, osteoporotic fractures are expected to have an increasing impact on the health of our population. Today, osteoporosis is estimated to affect over 20 million Americans with 1.5 million osteoporotic fractures occurring in the United States every year [1]. In women, the lifetime risk of experiencing a minimal trauma hip fracture, the most devastating consequence of osteoporosis, is estimated to be 17% [2]. In most patients with osteoporosis, the occurrence of a hip fracture is a devastating consequence, conveying 24% excess mortality within 12-months of the fracture and a continued increased risk of death for up to 10-years after the event [3]. In patients with the most advanced osteoporosis, currently available medications can, at best, decrease hip and non-spine fracture rates by 50% and 30%, respectively [4,5]. At present, the mainstay of osteoporosis treatment is the use of oral and intravenous bisphosphonates (BPs). These drugs act by suppressing bone resorption (as well as formation) and have been shown to increase bone mineral density (BMD) and reduce fracture rates in certain populations [6]. Teriparatide (TPTD- hPTH 1-34) has also been shown to increase BMD and reduce fractures, but acts by a different mechanism that involves stimulating new bone formation (along with resorption), and reconstituting internal bone microarchitecture [7-14]. The effects of TPTD are dose dependant both with regard to increases in bone mass and stimulation of osteoblast activity [13]. Specifically, biochemical markers of bone formation increase more in women treated with 40-µg of TPTD daily compared to women treated with 20-µg (the FDA approved dose) and this greater stimulation of bone formation is associated with greater BMD increases, particularly in trabecular bone [14,15]. Despite stimulating greater increases in bone mass, TPTD 40 mcg/day reduces spine and non-spine fractures no more than TPTD 20 mcg/day in humans. We hypothesize that this paradox arises because 40-mcg/day increases bone resorption more than 20-mcg/day, a difference revealed by biochemical markers of bone resorption [14,16]. Unique among osteoporosis medications, TPTD has also been shown to stimulate periosteal expansion in animal models though a similar property in human studies has yet to be unequivocally shown [8,17-19].

While both TPTD and BPs increase BMD and reduce fracture risk, their beneficial effects are limited and neither drug is able to completely normalize BMD or restore skeletal integrity in most patients. Initially thought to be a promising approach, trials of combination therapy with anabolic agents and BPs have demonstrated minimal or no advantage over monotherapy [20-26]. In these combination studies, the antiresorptive agent inhibited TPTD or PTH-stimulated increases in bone formation and resorption but did not suppress turnover to the degree observed with the antiresorptive agent alone [22-26]. Together, these studies suggest that co-administration of BPs with TPTD blunts the TPTD-associated stimulation of bone formation in a dose-dependent fashion, particularly in predominately trabecular sites such as the spine. The reasons that the combination of BPs and TPTD do not stimulate greater gains in BMD are unclear. BPs presumably interfere with one or more of the mechanisms via which TPTD exerts its anabolic effect on bone: increased secretion of IGF-I, bFGF, or other paracrine growth factors by osteoblasts, decreased production of sclerostin by osteocytes, and/or increased osteoclastic resorption of bone with resultant release of these and other pre-formed growth factors [23,27,28].

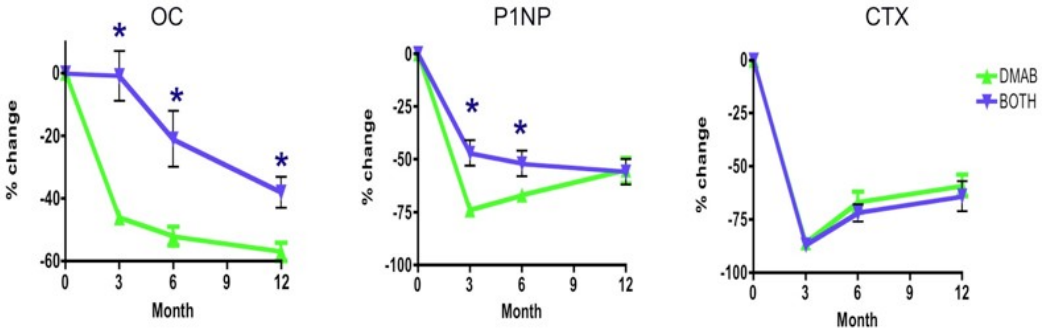
Rodent models have suggested that TPTD in combination with OPG (a molecule aimed at the same molecular target as denosumab– see below) has superior skeletal effects compared to TPTD alone [29,30]. Denosumab (DMAB) is an FDA-approved fully human monoclonal antibody that specifically binds to receptor activator of nuclear factor-κB ligand (RANKL)[31]. It inhibits bone resorption by interfering with the binding of RANKL to its receptor on pre-osteoclasts and osteoclasts, the final common pathway leading to osteoclast activation and bone resorption [32,33]. DMAB suppresses bone turnover more than intravenous and oral BPs [34,35]. DMAB's potent antiresorptive properties are best demonstrated in iliac crest biopsy specimens from postmenopausal osteoporotic women who had been taking the drug for 24-36 months. These biopsies revealed an 80% reduction in eroded surface

and the complete absence of osteoclasts in more than 50% of the specimens. Furthermore, histomorphometric indices of bone turnover were significantly lower in the patients taking DMAB than those taking alendronate [36]. Despite this dramatic suppression of osteoclast activity, however, bone turnover rapidly normalizes after the drug is discontinued [31]. Like other antiresorptive agents, DMAB increases BMD at the lumbar spine and hip, but unlike most of these agents also increases BMD at diaphyseal sites such as the wrist [37-42]. Furthermore, DMAB reduces the risk of vertebral and hip fractures in postmenopausal women with osteoporosis, and increases indices of bone strength when measured by HR-pQCT [43,44]. Taken together, the unique mechanism of action of DMAB and the suggestive animal studies led us to hypothesize that the combination of DMAB and TPTD would increase BMD, bone quality, and estimated bone strength more than either drug alone.

This hypothesis was tested and confirmed in The DATA Study (ClinicalTrials.gov Identifier NCT00926380) [45,46] that compared teriparatide and denosumab monotherapy to the combination of both drugs in postmenopausal osteoporotic women. As shown below, the results not only showed that the combination increased hip and spine BMD more than either drug alone, but also that in the presence of DMAB, the pro-resorptive effects of TPTD are completely blocked (unlike combinations of TPTD and BPs).



The combination group is statistically different from both other groups at all sites (P<0.02). TPTD group is statistically different from DMAB at total hip only



*For clarity, changes are shown for combination and DMAB groups only (*P=0.05 for between-group comparison). In the TPTD alone group, typical increases in OC, P1NP (bone formation markers), and CTX (bone resorption marker) were observed.*

These results suggest that even greater gains in BMD, bone quality, and bone strength could be achieved if DMAB could be combined with a more potent stimulant of osteoblast function and bone formation. Thus, we plan to conduct 15-month study that compares TPTD 40-µg plus DMAB (60-mg every six months) to TPTD 20-µg plus DMAB in a design that also takes advantage of recent work that suggests that starting TPTD before the antiresorptive has some clinical advantages [47,48].

In performing this clinical trial, we will be able to better understand the mechanisms by which antiresorptive agents influence the efficacy of anabolic agents. Furthermore, if our hypothesis is confirmed, the findings of this study could define a novel approach to the treatment of high-risk osteoporosis patients.

A known limitation of both teriparatide and denosumab therapy is the rapid loss of bone that occurs when these drugs are discontinued.[49,50] Moreover, both teriparatide and denosumab are expensive and administered by subcutaneous injection (daily and every 6-months, respectively). If combination teriparatide/denosumab therapy is to become part of a widely used treatment approach, we must develop strategies to maintain and expand the unprecedented improvement in bone density and microarchitrecture observed, and do so with acceptable risk of serious adverse effects. Zoledronic acid is a highly potent bisphosphonate that has been shown to reduce the risk of both vertebral and non-vertebral fractures when given as a yearly 5-mg infusion over 3 years.[51] Despite the FDA-approved yearly dosing schedule, it has also been reported that the effects of a single 5-mg zoledronic acid infusion are much more prolonged. Specifically, a single administration of zoledronic acid continues to reduce bone resorption and increase BMD for an additional 2-5 years.[52-55] Moreover, it has also been demonstrated that a single dose of zoledronic acid continues to reduce fracture rates for at least 3-years and to the same degree as do yearly infusions.[56]

As the optimal treatment for patients who have previously received combination denosumab and teriparatide is not defined, we believe that is useful and important to look at how our study subjects' bone mineral densities have changed a year or more after completion of their participation in our study.

A secondary substudy will explore the effects of the different combination treatments on bone material strength as measured by reference point indentation. In vivo reference point indentation measures was shown to discriminate between patients with and without fragility fracture (50). The objective of this substudy is to assess change in bone quality using this new minimally invasive procedure. Longitudinal studies with reference point indentation are limited and no published studies have assessed the bone material strength changes as measured by reference point indentation in response to the combination of antiresorptive and anabolic therapy.

II. Specific Aims

The Specific Aim of this protocol is to test the following hypothesis:
In postmenopausal women with osteoporosis, the combination of DMAB and high-dose TPTD (which will be started 3-months prior to denosumab) will increase BMD, improve bone microarchitecture, and increase estimated bone strength more than the combination of DMAB and standard dose TPTD.

The Specific Aim of the substudy is to test the following hypothesis:
In postmenopausal women with osteoporosis, the combination of DMAB and high-dose TPTD will improve bone material strength more than the combination of DMAB and standard dose TPTD.

The Specific Aim of the Extension trial (DATA-2-EX):
The extension trial described (DATA-2-EX) will test the hypothesis that the large increases in cortical and trabecular BMD, bone quality, and estimated bone strength achieved with combined DMAB and high-dose TPTD therapy can be extended for up to 27-months by a single 5-mg dose of zoledronic acid. If this hypothesis is confirmed, it will represent a significant advance in the rational development of comprehensive treatment strategies, especially for those with severe disease in whom single-agent therapy does not adequately reduce fracture risk. Additionally, positive results from DATA-2-EX would support the initiation of a larger trial assessing the anti-fracture efficacy of this approach.

The Specific Aim of the Observational visit:

This optional observational visit is specific to subjects who completed the month-15 trial but did not enroll in DATA-2-EX. We wish to test the hypothesis that further transitioning to intravenous bisphosphonate from the two treatment groups will prevent the bone loss associated with discontinuing these therapies and will further increase bone density.

III. Subject Selection

350 female volunteers will be screened and 70 female volunteers will be recruited by advertisement in accord with institutional guidelines for clinical studies. Recruitment flyers will be posted in approved locations throughout the MGH (including the MGH Bone Mineral Density Center) and email announcements will be sent through the Partners Clinical Research Program Network. Letters will be sent to subjects identified through RSVP for Health. Additionally, advertisements will be published in local newspapers and a mailing will be sent to targeted populations, including subjects who have expressed an interest in one of our research group's previous studies.

Subjects will be paid $50 after each completed visit (other than the screening visit) for a total of $300.

Parking vouchers will be provided to subjects at each visit, including the screening visit.

Inclusion Criteria:

Must satisfy A and B and C and D below:

A. women aged ≥ 45

B. postmenopausal by either of the following criteria:

> 36 months since last spontaneous menses;
or
> 36 months since hysterectomy, plus serum FSH ≥ 40 units / liter.

C. osteoporosis with high risk of fracture' by one or more of the following criteria:

DXA spine or hip T-score ≤ - 2.5;
or
DXA spine or hip T-score ≤ - 2.0 plus ≥ 1 of the following BMD-independent risk factors for fracture: fracture after age 50, parental hip fracture after age 50, prior hyperthyroidism, inability to rise from a chair with one's arms elevated, current tobacco smoker.
or
DXA spine, hip, or forearm T-score ≤ - 1.0 plus ≥ 1 adult low-trauma fracture (low-trauma fracture = fracture after no trauma; or fracture after falling ≤ 6 inches when stationary or moving slower than a run.

Exclusion Criteria:

- confirmed serum alkaline phosphatase above upper normal limit with no explanation
- liver disease (AST or ALT > 2 x upper normal limit).
- renal disease (serum creatinine > 2.0 mg/dl).
- Hypocalcemia or hypercalcemia (Ca <8.5 mg/dL or >10.5 mg/dL)
- Abnormal blood PTH (intact PTH < 10 pg/mL or > 65 pg/mL)
- serum 25-OH vitamin D < 20 ng/ml
- HCT < 32%.
- history of malignancy (except non-melanoma skin carcinoma) or radiation therapy.
- history of gouty arthritis.
- significant cardiopulmonary disease including unstable coronary artery disease, stage D ACC/AHA heart failure or any other condition that the investigator deems may preclude the subject from participating safely or completing the protocol procedures.

- major psychiatric disease that in the opinion of the investigator would preclude the subject from providing adequate informed consent or completing the protocol procedures.
- excessive alcohol use or substance abuse that in the opinion of the investigator would preclude the subject from providing adequate informed consent or completing the protocol procedures.
- known congenital or acquired bone disease other than osteoporosis (including osteomalacia, hyperparathyroidism, Paget's disease)
- current use or use in the past 6 months of oral bisphosphonate
- current use or use within the past 3 months of estrogens, selective estrogen receptor modulators, or calcitonin.
- Current use or use in the past 6 months of denosumab
- use of oral or parenteral glucocorticoids for more than 14 days within the past 6 months
- any current or previous use of strontium, teriparatide, or any parenteral bisphosphonate.
- known sensitivity to mammalian cell-derived drug products.
- known sensitivity to teriparatide or any of its excipients.
- known sensitivity to densoumab or any of its excipients.
- Extensive dental work involving extraction or dental implant within the past 2 months or planned in the upcoming 2 months.

Microindentation substudy criteria:
All subjects eligible for the main study are eligible for the microindentation substudy.

DATA-2-EX criteria:
Subjects completing DATA-2 will be offered enrollment in DATA-2-EX if they meet the same entry criteria outlined in DATA-2 with the following exceptions related to fracture risk. Subjects currently enrolled will be offered enrollment in DATA-2-EX by letter and a follow-up phone call at least 2 months prior to their 4th study visit. Subjects being recruited will be informed of the optional extension during the screening phone call.

All subjects enrolled in DATA-2 will be eligible for DATA-2-EX or the optional observational visit.

In addition to these DATA-2 criteria, to be enrolled in DATA-2-EX, women will be required to have an estimated GFR $\geq$35 (mL/min), a 25-(OH) Vitamin-D level $\geq$20 (ng/mL), and no known allergy to bisphosphonates. Laboratory testing of Cr, GFR and 25-OH vitamin D will be performed within 3 months of receiving zoledronic acid. The exclusion criteria will remain the same as the original DATA-2 exclusion criteria, as outlined above, with the exception that prior exposure to denosumab and teriparatide as part of the DATA-2 protocol are permitted and renal function criteria will be assessed by estimated GFR (rather than the creatinine level). Zoledronic acid will not be infused if the 25-OH vitamin D level is <20 ng/mL or GFR is <35 mL/min.

IV. Subject Enrollment

This outpatient study will either be conducted at one or more of the following sites: the General Clinical Research Center (GCRC), the Osteoporosis Research Center or the Endocrine Unit Clinical Space (all within the Massachusetts General Hospital.

The general design is a randomized, 2-arm, open label study. Participation in the microindentation substudy will be optional.

Groups and Randomization
Subjects will first be screened, and those who appear eligible will have a medical history and interview with a study investigator. Those who remain eligible will be formally accepted into the study by a study investigator, assigned to one of 4 strata based on age (above or below 65) and previous bisphosphonate use (previous use or none) and then randomly assigned within each strata to one of the 2 treatment groups by computer-generated cards. (1:1: ratio). Within each of the four strata, the randomization block size will vary randomly to minimize the predictability of treatment assignments.

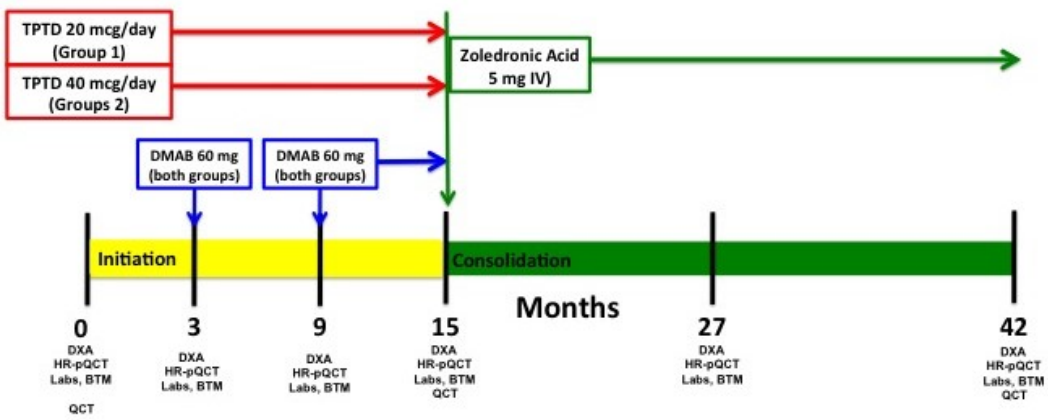
The study period lasts 15 months. There will be 35 subjects in each group.

All subjects completing the 15-month DATA-2 trial be offered enrollment in the DATA-2-EX study.

For subjects who do not enroll in DATA-2-EX, subjects may enroll for an optional observational visit. The observational period will start immediately after the DATA-2 study's 15-month visit and will last an additional 12 to 24 months. During that interval, each subject will be contacted by phone.

All subjects will be asked if:
- They continued osteoporosis treatment and if so, with what drug
- They have sustained any new fractures.

We will request that all subjects return for one visit to complete one bone density scan of the spine and hip (by DXA) within 12 to 24 months of the completion of the 15-month study. Informed consent for this visit will be obtained by a research assistant. The DXA will be performed at no cost to the study subjects.



Note: (Optional one-time observational visit for only DXA may occur between month 27 and month 39 for subjects who do not receive zoledronic acid.)

All subjects who report dietary intake of less than 1200mg of calcium daily will be given calcium supplementation (600mg elemental calcium) with Vitamin D (400IU). Subjects with a dietary intake > 1200 mg will receive Vitamin D only.

V. Study Procedures

Telephone screening and Screening Visit:
Members of research staff will screen all interested subjects over the telephone. If subjects meet initial criteria and are interested in the study, a member of the study staff will schedule a screening

visit wherein the subject will sign a separate “screening only” consent form. This consent form will allow for a blood draw and bone mineral density testing.

Prior to signing this screening only consent form, subjects will be offered the option to speak with a physician investigator if they wish or if they have questions that can't be answered by the RA.

At this visit, bone mineral density of the spine and hip will be obtained and the following will be measured in serum or blood:

- PTH
- 25-(OH) Vitamin D
- Routine chemistries (Calcium, Phosphate, electrolytes, BUN, creatinine, ALT)
- Complete Blood Count
- FSH (if necessary because of prior hysterectomy)

Visit 1:
If the subject meets the above BMD and laboratory criteria, she will then be scheduled for visit 1. At this visit, a study physician will obtain written informed consent for participation in the complete study. If subjects require more time to consider participating after reading the consent form the screening visit will not continue. If the subject is ready to sign the consent form, the study physician will then perform a history and physical on her. If all inclusion/exclusion criteria are met, subjects will then continue visit 1 and the subsequent visits.

Visit 1-6:
These visits will occur at the following times:
Visit 1 (month 0)
Visit 2 (month 3 +/- 2 weeks)
Visit 3 (month 9 +/- 2 weeks)
Visit 4 (month 15 +6/- 2 weeks)
Visit 5 (month 27 +/-2 weeks)
Visit 6 (month 42 +/-2 weeks)

If enrolled in optional observational study, these visits will occur at the following times:
Visit 1 (month 0)
Visit 2 (month 3 +/- 2 weeks)
Visit 3 (month 9 +/- 2 weeks)
Visit 4 (month 15 +/- 2 weeks)
Visit 5 (month 27, up to month 39) Visit 5 will consist of obtaining informed consent and DXA hip and spine scan only. A blood draw will not be performed.

Subjects will be seen at MGH prior to 10 a.m. and will be instructed to be fasting, and to skip that day's PTH injection (if any). The following blood/serum tests will be measured:

- Routine chemistries (Calcium, Phosphate, electrolytes, BUN, creatinine, ALT, PTH, 25OH vit D)
- Complete Blood Count
- Serum CTX (pooled assay of de-identified samples at end of study to be analyzed by collaborator Dr. Richard Eastell)
- Serum osteocalcin (pooled assay of de-identified samples at end of study to be analyzed by collaborator Dr. Richard Eastell)
- Serum PINP (pooled assay of de-identified samples at end of study to be analyzed by collaborator Dr. Richard Eastell)

After which the subjects will be given breakfast, and then have the following procedures per table below:

The following procedures are performed at each visit:

	Screen	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Month		**0**	**3**	**9**	**15**	**27**	**42**
Routine and hormonal lab testing	X	X	X	X	X	√	√
History and Physical Exam	X				√		
Dispense Medications		X	X	X	√		
Bone Turnover Markers		X	X	X	X	√	√
BMD by DXA of PA spine, hip, and 1/3 distal radius	X	X	X	X	X	√,*	√
HR-pQCT of radius and tibia		X	X	X	X	√	√
QCT of the hip and spine		X			X		√
Microindentation (for those in the substudy)		X	X		X		

X = DATA-2 procedures
√ = DATA-2-EX procedures
*= observational visit, DXA spine and hip only

After blood sampling, denosumab (sold under the brand name Prolia) will be administered (visits 2 and 3 only). Additionally, at visit 1 subjects will be trained in the use of the teriparatide pen (sold under the brand name Forteo®) and will give themselves the first injection while observed by a member of the study staff. At each visit participants will return their used and unused Forteo® injection pens, so we can estimate and record the unused volume in each pen and thereby estimate her compliance with Forteo® use. All subjects in DATA-2-EX receive a single 5-mg infusion of zoledronic acid at month 15.

Study funds and NIH funding will cover the costs of all study procedures, ancillary medications and the costs of the study drugs if not covered by insurance or the manufacturer.

Substudy:
Bone indentation procedure (adapted from Diez-Perez et al Journal of Bone and Mineral Research 2010): The bone indentation device [57] is made by ActiveLife Scientific (Santa Barbara, CA), and has been used in several clinical studies [58,59]. Briefly, after local anesthesia with 2% lidocaine and cleaning of the skin over the midshaft of the tibia with ChloraPrep, the sterile probe assembly will be inserted through the skin to rest on the bone surface. Subsequently measurements will be actuated with indentations of the bone surface with a maximum force of 10 N. These measurements will be repeated up to 10 times separated by at least 2 mm from other measurement locations. After the final measurement a bandage is applied. The indentations are small, on the order of 375 μm across — equivalent to the width of a human hair. Measurements are made on the non-dominant leg. Microindentation will be performed at key changes in treatment at months 0, 3, and 15.

Sterilization of reference probes: Unsterilized reference probes are shipped to investigators from the manufacturer. Upon receipt, probes are individually packed into self-sealing autoclave pouches with sterilization indicators. Pouches are sterilized in an autoclave certified and maintained by MGH.

Pouches are visually inspected to ensure adequate sterilization, and dates of sterilization are recorded. A new probe is used for each subject.

Replacement of reference probe indentation device: The device (Osteoprobe) does not need to be replaced. A new sterile reference probe is inserted into the device for each patient.

** We note that the bone microindentation procedure has been previously approved by the Partners IRB, Protocol #2013P001051, PI: Dr. Mary Bouxsein.

Note: Any remaining serum samples will be saved and may be used to measure other variables related to bone metabolism at a future time. Samples will be stored with subject code number and date and time of sampling in a locked –80 degree freezer and only approved study personnel will have access to the samples. It is understood that any future use of samples must be reviewed and approved separately by the HRC prior to their use. Personal-Identifying information will be removed before any sample is sent out for analysis to a non-Partners affiliated institution.

Note: The Principal Investigator may end the subject's participation without the subject's consent in order to protect the health of the subject, if the subject is unable to attend to study visits, if the sponsor decides to stop the study, or due to other administrative reasons.

Interpretation of results and study limitations of the proposed clinical trial:
This 15-month RCT will provide scientifically important and potentially groundbreaking clinically relevant findings. The combination of anabolic therapy with BPs has not been promising but the DATA study demonstrates that DMAB, through its distinct mechanism of action, may prove to be a more effective inhibitor of osteoclast activity in the presence of a potent anabolic stimulus. By increasing the TPTD dose, we hypothesize that we will maintain or improve bone formation rates while resorption remains powerfully inhibited. If the combination of this higher TPTD dose and DMAB increases BMD, improves bone quality, and increase estimated bone strength more than the combination of high-dose TPTD and alendronate and the combination of standard-dose TPTD and DMAB, it will represent a significant step forward in osteoporosis therapy. Additionally, analysis of the QCT and HR-pQCT assessments will potentially confirm the robust effects of DMAB and TPTD co-administration on the cortical compartment of the axial and appendicular skeleton.

VI. Study Endpoint and Statistical Analysis

The primary endpoint is the change in PA spine BMD (DXA) between treatment groups at 15 months. The method of analysis will be a longitudinal mixed effects ANOVA. This model includes the subject level random intercept, time, fixed treatment effect, and the (group)X(time) interaction term. The inference of the regression coefficient associated with the interaction term (difference in slopes) will determine differences between treatment groups. The model will allow us to include all available partial observations for the non-completers and this approach will provide more valid estimates (with increased efficiency) of the time effects than the last value carried forward method.

Calculation of Power
For purposes of estimation, we calculated the sample based on a between-group comparison of the mean intra-individual treatment effect over the treatment period for the primary and secondary endpoints based on the our observations of the standard deviation of the 12-month treatment effects observed in the DATA study. We predict that 30 subjects in each group will complete the study. This represents an 86% completion rate, a conservative estimate based on our current withdrawal rate of less than 6% in DATA. With this group size, the table below shows our estimated power calculations using the Hsu (With Best) multiple comparison test at a 5% significance level and assuming 80%

power. The Hsu completers-based power analysis is conservative, and the actual analysis will likely attain greater power as the full analysis set can be utilized.

Parameter	Standard Deviation of 12-month change in subjects receiving combination therapy in DATA.	Minimum detectable difference at a 5% significance level assuming 80% power
DXA PA Spine (primary endpoint)	**3.8%**	**2.5%**
DXA Fem. Neck	4.3%	2.8%
DXA Total Hip	2.8%	1.9%
Distal Radius Total vBMD	2.2%	1.5%
Distal Radius HR-pQCT Cortical vBMD	1.5%	1.0%
Distal Radius HR-pQCT Cortical Thickness	4.5%	2.9%
Distal Tibia Total vBMD	2.1%	1.4%
Distal Tibia HR-pQCT Cortical vBMD	1.5%	1.0%
Distal Tibia HR-pQCT Cortical Thickness	3.1%	2.4%
CTX	35%	23%
Osteocalcin	27%	18%
P1NP	32%	21%
Bone Material Strength measure (substudy)*	8.8 BMS units	6.5 BMS units

These detectable between-group differences are at the lower limit of what is considered clinically important and generally correspond to the between-group difference that we observed in the DATA study.

* *Derived from control group SD as reported in ref [60].* The difference in BMS units that we have power to detect is lower than the reported change in BMS after only 7-weeks of TPTD monotherapy in patients receiving glucocorticoids (55).

VII. Risk and Discomforts

Denosumab

DMAB is an FDA approved medication used to treat osteoporosis in various populations, including postmenopausal women. More than 13,500 patients have been treated with DMAB in clinical studies, and it is generally well tolerated. In clinical studies, it has been reported that DMAB may uncommonly produce the following side-effects: eczema, serious skin infections, low blood calcium (which can cause tingling in the fingers or around the mouth, muscle cramps, or abnormal heart rate), pain in the joints or extremities, high blood cholesterol, dizziness, cough, difficulty emptying the bladder, and decreased skin sensation. Hypocalcemia has been very rarely reported in absence of renal failure. Osteonecrosis of the jaw has been reported in the oncology clinical trial program in patients treated with DMAB but its relative incidence compared to placebo is unclear**.** Atypical, low-energy, or low trauma fractures of the femoral shaft have been reported patients who received DMAB. The incidence of atypical femur fractures is not well-defined but appears to be extremely rare. If a study subject presents with thigh or groin pain, she will be immediately evaluated to rule out an incomplete femur fracture.

Teriparatide

TPTD is an FDA approved medication used to treat postmenopausal women with osteoporosis who are at high risk of fracture. The most commonly reported side effects of TPTD are overall pain, asthenia, nausea, headache, leg cramps, sinus tachycardia, arthralgia, rhinitis), and dizziness. TPTD can cause transient hypercalcemia, which is generally mild, even with the 40-µg dose [13]. Repeated daily administration of TPTD in high doses to rodents causes dose-dependent osteosclerosis, bony obliteration of the marrow space, and extra-medullary hematopoiesis, followed by osteosarcomas. The relevance of these findings to humans is unclear and will remain so for many years, because osteosarcoma is rare in adults (4 cases/million/year). Eli Lilly and the FDA are monitoring the incidence of osteosarcoma in TPTD treated patients and have not reported a linkage thus far.

The dose of TPTD chosen (40-µg) is higher than that currently approved by the U.S. Food and Drug Administration (20-µg once daily) but the same as doses used in other clinical studies, including our own. Studies from our group were performed under an IND to Dr Robert Neer (co-investigator) and include the following:

Study	Population	Total subjects receiving TPTD 40-µg SC daily	Duration	Hypercalcemia incidence (24-hrs post-dose)
Finkelstein et al. NEJM 2003 [24]	men	55	24 months	<1%
Finkelstein et al. JCEM 2010 [23]	postmenopausal women	62	24 months	<2%

In the largest study of the 40-µg dose of teriparatide [13], 541 women received the 20-µg dose and 544 women received the 40-µg dose for a mean if 19 months. In this study, both doses were generally well tolerated and differences in side effects between doses were small. Side effects included nausea (40-µg 18%, 20-µg 8%, placebo 8%), headache (40-µg 13%, 20-µg 8%, placebo 8%), dizziness (40-µg 6%, 20-µg 9%, placebo 6%), and leg cramps (40-µg 1%, 20-µg 3%, placebo 1%). Additionally, the 40-µg dose was well-tolerated in a group of 122 postmenopausal women when co-administered with hormone replacement therapy for 24 month [15].

Zoledronic Acid

The most common side effects associated with zoledronic acid include flu-like symptoms (e.g., fever, chills, muscle/joint aches) occurring after the infusion. The majority of these symptoms occur within the first 3 days following drug administration and usually resolve within 3 days of onset but resolution can take up to 7-14 days. Taking acetaminophen or ibuprofen after the infusion can mitigate these symptoms. Other common side effects include nausea, tiredness, dizziness, headache, or pain/redness/swelling at the injection site.

Renal toxicity: Zoledronic acid, has been associated with renal impairment and in rare cases, acute renal failure. There is a transient increase in serum creatinine within 10 days of dosing in 1.8% of zoledronic-acid treated patients versus 0.8% of placebo-treated patients. Renal function is being monitored closely in both DATA-2 and DATA-2-EX and patients will be required to have an estimated GFR >50 (mL/min) to receive zoledronic acid 5mg. Subjects with GFR 35-50 would receive zoledronic acid 2 mg. As per the approved product labeling, the infusion time for zoledronic acid will be at least 15 minutes and will be followed by a 10 mL normal saline flush of the intravenous line.

Hypocalcemia: In clinical trials of zoledronic acid without Vitamin D pre-treatment, approximately 0.2% of patients had notable declines of serum calcium levels to values less than 7.5 mg/dL after each zoledronic acid administration. No symptomatic cases of hypocalcemia were observed in clinical trials if they had been pretreated with Vitamin D. All patients in DATA-2-EX are being treated with Vitamin D and Vitamin D levels are being monitored in both DATA-2 and DATA-2-EX. Patients are required to

have a pre-treatment 25-(OH) Vitamin-D level ≥20 (ng/mL). Patients who are administered the renal dose of zoledronic acid (2mg) will be asked to return for bloodwork 10 days (+/- 3 days) post-infusion to assess calcium and creatinine levels.

Osteonecrosis of the Jaw: Osteonecrosis of the jaw is an extremely rare side effect of zoledronic acid when given at the doses recommended for osteoporosis treatment. Subjects will not be enrolled in DATA-2-EX if they have any major dental work planned during the projected course of the study (extractions or implants). An oral examination will be performed by a study doctor prior to administration of zoledronic acid.

Atypical Femur Fractures: Atypical, low-energy, or low trauma fractures of the femoral shaft have been reported in bisphosphonate-treated patients. Atypical femur fractures may be unilateral or bilateral and some patients report prodromal dull or aching pain in the thigh in the weeks to months before a complete fracture occurs. The incidence of atypical femur fractures is not well-defined but appears to be extremely rare and at least somewhat dose-dependent. If a study subject presents with thigh or groin pain, she will be immediately evaluated to rule out an incomplete femur fracture.

Imaging studies

Over the 15-month study period, subjects will receive the following imaging studies:

Study Type	DATA-2	DATA-2-EX	Radiation exposure per scan
DXA of the lumbar spine, hip, and radius.	5 (including screen)	2	1 mrem
HR-pQCT of the distal tibia and radius	4	2	<0.5 mrem
QCT of the hip and spine	2	1	510 mrem

The total dose for the DATA-2 15-month study is therefore ~1000 mrem . The total dose for DATA-2-EX (months 15-42) is ~500 mrem.. This amount of radiation is equal to the background radiation one is exposed from the earth and sky over 3.25 years (for DATA-2) and 1.5 years (for DATA-2-EX).

The total dose for the observational visit alone is 0.05 mSv, equal to background radiation one is exposed from the earth and sky over 3 days.

Microindentation substudy

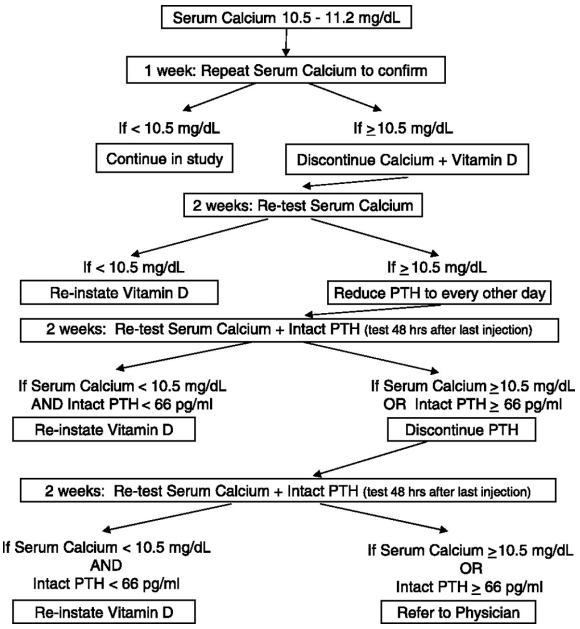
Subjects may feel some pain when the local anesthetic is given to numb the testing area on their shin bone. Some people (fewer than 1 in 10,000) are allergic to the lidocaine. A severe allergic reaction (anaphylaxis) to lidocaine, although rare, may possibly be life-threatening. Also, a burning sensation at the spot where the lidocaine is injected may occur. Other risks of lidocaine include: low blood pressure, nausea, and slow and/or irregular heartbeat. Subjects may have a bruise (black-and-blue mark) or pain where we perform the bone indentation test. There is also a small risk of feeling lightheaded, fainting, or infection. There may be other risks of the bone strength test that are unknown at this time.

VIII. Potential Benefits

The medications given in this study have been shown to reduce fracture risk as well or better than all other approved osteoporosis therapies and subjects can expect to derive the benefit from these medications. It is hoped that the knowledge gained from this study will improve osteoporosis care.

IX. Monitoring and Quality Assurance

A study physician will review all laboratory test results. Subjects will be discontinued from the study if they have any finding that in the opinion of the study physician requires withdrawal from the study. Disqualifying findings will be communicated to both the subject and her primary care physician. If the subject does not have a primary care physician, the principal investigator will coordinate appropriate follow-up with a physician of the subject's choosing. Hypercalcemia is a well-described complication of teriparatide treatment. Elevated blood calcium levels will be handled in the manner recently recommended by Antoniucci et al (see below) [61].



If serum calcium concentration is above 11.2 mg/dl, teriparatide will be stopped and serum calcium measured at least daily until normal. Thereafter teriparatide will be resumed every other day and the above algorithm followed, starting at "Reduce PTH to every other day".

The Data Safety Monitoring Board (DSMB) that is currently monitoring DATA 1 will monitor DATA 2. This DSMB is comprised of experienced clinical investigators: Dr. Deborah Wexler (Chair), Dr. Carl Pallais, and Douglass Hayden (biostatistician). Adverse events and serious adverse events will be reported to all appropriate regulatory bodies as required by current regulations.

A NIAMS-appointed DSMB will provide safety oversight for DATA-2-EX. This DSMB will meet as per NIAMS/KAI guidelines. As per NIAMS reporting requirements, all adverse events will be reported in the routine safety reports and all serious adverse events regardless of relatedness will be reported to the DSMB Safety Officer and the NIAMS, through KAI (NIAMS Executive Secretary), within 48 hours of the PI receiving notification of the events.

Adverse events or other unanticipated problems will be reported to the Partners Human Research Committee (PHRC) within 7 calendar days/5 working days of the date the investigator first becomes aware of the problem as described in the PHRC policy on Adverse Event Reporting and Unanticipated Problems Involving Risks to Subjects or Others.

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