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Title: Pilot Study of the Effect of High Doses of Radiation on Bone Metabolism and Structure in Patients Treated with Adjuvant Radiotherapy and Surgery for Sacral Tumors.

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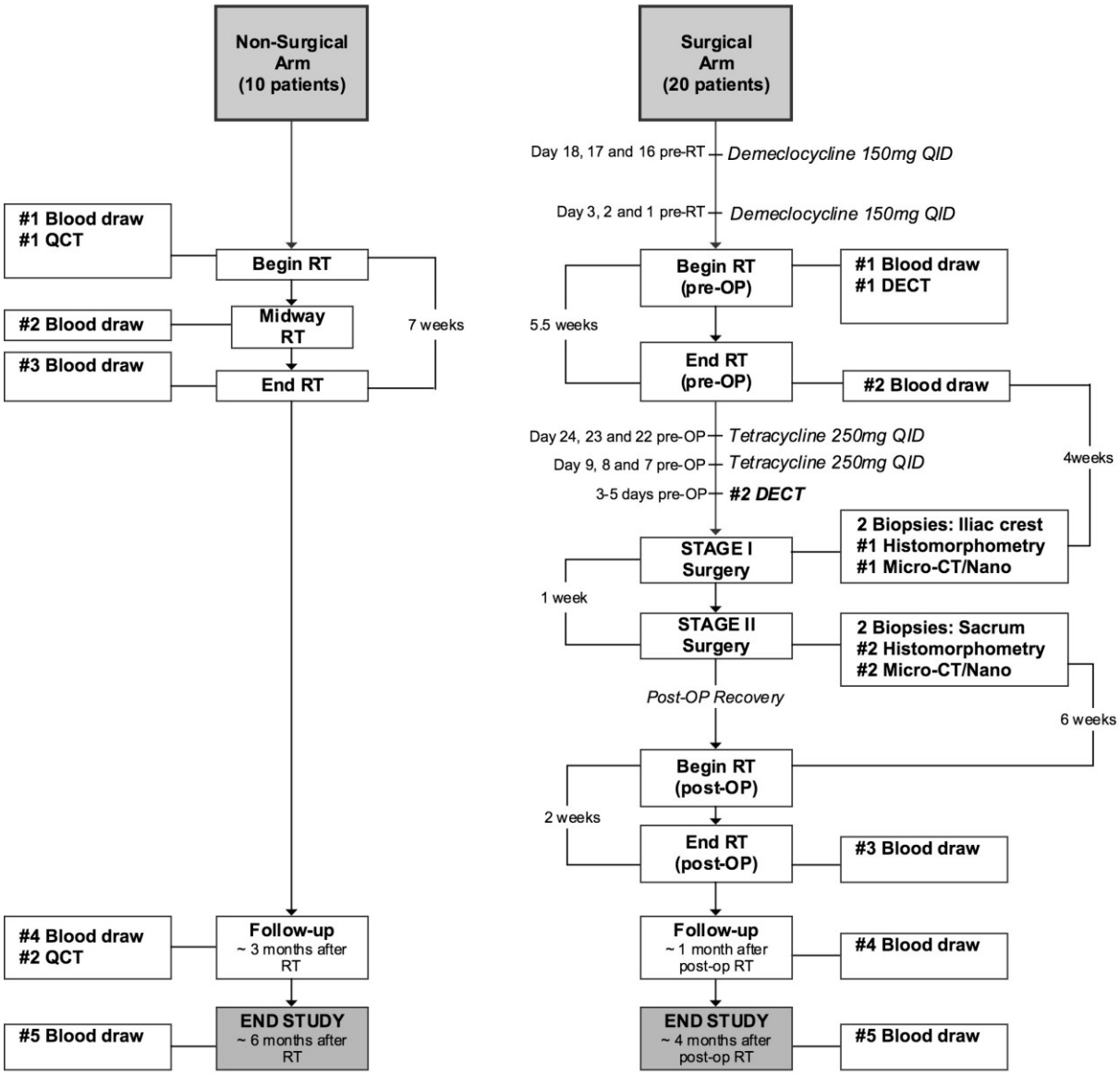
SCHEMA

Note:
* A window of up to +1 week is allowed for the interval between end of radiation and surgery (stage I surgery can be performed 4 to 5 weeks after completion of radiation).
** 6 weeks is an average interval between surgery and start of radiation. A window of +/- 2 weeks is allowed for the interval between surgery and postoperative radiation to allow adequate time for wound healing. #3 Blood draw can take place whenever postoperative radiation has been completed.



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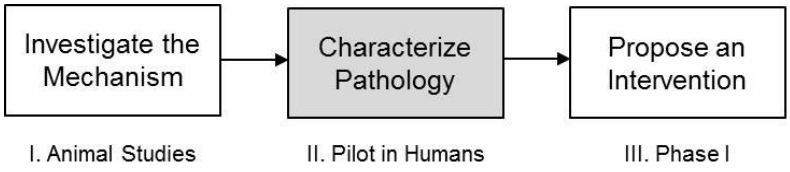
1. OBJECTIVES

1.1 Study Design

This is a pilot study designed to characterize the effects of high energy radiation on human bone degradation, with a specific interest in reducing the rate of sacral fractures.

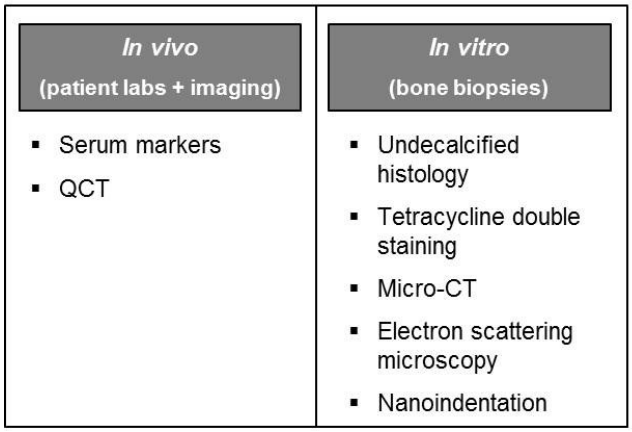
Figure 1: Phase Transition into Clinical Trials



Based on the data published in animal models, we hypothesized that bone degradation after irradiation is associated with clinically detectable changes in serum markers, bone density, and bone architecture. This study is a step towards better characterizing those changes in humans, and understanding the mechanism of the underlying pathology. The results of this research may ultimately lead to the development of interventions aimed at reducing the burden of radiation-induced fractures, as outlined in Figure 1:

Figure 2 provides a succinct layout of the proposed experimental plan and a summary of the data we expect to collect.

Figure 2: Experimental Design Summary



Experimental Design. We plan to conduct *in vivo* and *in vitro* studies to characterize radiation-induced changes in bone. We will look at changes in the cellular composition of bone, bone metabolism, microscopic architecture, and mechanical properties.

1.2 Primary Objectives

1.2.1 To measure the effect of high doses of radiation on trabecular bone mineral density (Tb. TBD) in the adult sacrum using the quantitative computer tomography technique (QCT) in patients undergoing treatment with combination surgery and adjuvant radiotherapy, or radiation alone.

1.3 Secondary Objectives

1.3.1 To characterize changes in the levels of serum markers of bone turnover during the course of 12 months following treatment with high doses of radiation

1.3.2 To analyze the effects of high doses of radiation on the structure and mechanical properties of bone by microscopic computer tomography (micro-CT), and nanoindentation

1.3.3 To characterize the effect of high doses of radiation on the dynamic parameters of bone turnover (e.g. mineral apposition rate, mineral formation rate, and mineralization lag time) by tetracycline quadruple labeling method

1.3.4 To characterize the effect of high doses of radiation on the mineralized and cellular components of bone architecture (e.g. volume, thickness, separation of trabecular bone, and osteoid surface and volume) by undecalcified bone histology

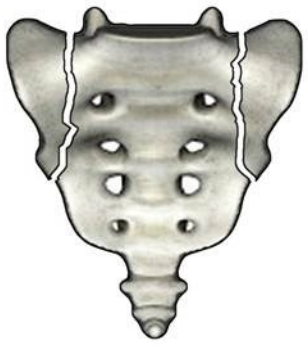
2. BACKGROUND

2.1 Rationale

External beam radiation plays an important role in the management of bone malignancies. In combination with surgical resection, high dose radiation was shown to significantly improve survival in historically difficult to treat tumors [4, 6, 15, 22]. The detrimental effects of radiation increase proportionally with the dose delivered. Even at relatively low doses of radiation (30 to 40 Gy), skeletal complications such as radiation-induced osteoporosis, insufficiency fractures (see

Figure 3), physeal arrest, and non-union are common [2, 14, 21, 29, 33]. At high doses (70 Gy and above), the rate of bone-related complications increases dramatically. Despite the high rate of complications, high-dose radiation is becoming increasingly more utilized because of its positive effects on patient survival and prevention of recurrence. For example, combination of surgery and adjuvant radiation doses of greater than 70 Gy achieves meaningful control of locally aggressive tumors (like sacral chordomas), that otherwise have a very high rate of recurrence [6].

Figure 3: Bilateral Sacral Fractures



Sacral Insufficiency Fractures. Schematic representation of the bilateral insufficiency fractures through the sacral ala. Unfortunately, this type of fracture is not uncommon among patients treated with high doses of radiation. It can cause debilitating back pain, and can be quite detrimental to the recovery process.

Given its growing significance in the treatment of malignant bone tumors, we propose to investigate the impact of high dose radiation (over 70 Gy) on human bone metabolism, architecture, and mechanical properties. It is presently unknown what biological mechanisms and structural changes occur in human bone in response to high doses of radiation. Local effects on bone may differ for different parts of the body. To minimize the variability in bone properties, we have chosen to focus this study on one type of bone - the sacrum. Still, our results are likely to be generalizable to other bones because of the common physiology. Sacrum is a particularly good target for this study because 1) tumors affecting the sacrum are often treated with high doses of radiation and 2) sacrum is one of the bones maximally affected by post-radiation complications**.**

Figure 3 shows a schematic representation of a typical insufficiency fracture that can occur in the sacrum after radiation treatment. Our group recently reported the rate of sacral insufficiency fractures as high as 64% in patients treated with high dose radiation and surgery (submitted). Thus, for the purposes of this pilot study we will focus on patients with primary malignant tumors of the sacrum.

Most MGH patients with sacral tumors are treated with combination of surgery and adjuvant radiation. They receive 50.4 Gy of photon/proton beam radiation before surgery, in accordance with the protocol summarized in Figure 9 (A). After the tumor is surgically removed, patients receive another 19.8 Gy of radiation, for a total dose of 70.2 Gy. We plan to enroll 20 patients treated in this fashion into the "Surgical Arm" of the study. It has been previously suggested that in patients treated with combination of surgery and radiation, the high rate of sacral fractures may be partially attributable to surgery. Yet, sacral fractures have been reported in patients who were treated with radiation alone. There is no data to date that can account for these observations. To explore this question, we plan to enroll 10 patients treated with radiation alone into the "Non-surgical Arm" of our study. These patients receive between 72 Gy

(osteosarcoma and chondrosarcoma) to 77.4 Gy (for chordoma) of radiation over the course of 7 to 8.5 weeks, as outlined in Figure 9 (B).

The long term goal of this research is to improve treatment outcomes and quality of life in patients with tumors of any origin, who undergo treatment with radiation. We hope that the results of this translational research may ultimately enable the predication of patients at risk of fracture and allow intervention before fracture occurs.

2.2 Study Disease

This study does not focus on a single disease. Instead, the study is concerned with effects of high dose radiation therapy on bone quality in general. For the pilot study, we will focus on patients with primary malignant bone tumors of the sacrum, but the results will most likely be generalizable to other conditions, including metastatic disease.

2.3 Bone Labeling Drugs

2.3.1 *Tetracycline*

Tetracycline is an FDA-approved anti-microbial drug, available in the US in generic form. It has minimal, well-documented adverse effects. Tetracycline can be safely self-administered by the patient in a form of oral pill on outpatient basis. In this study, it will be used as a fluorescent label for dynamic bone histomorphometry studies, in accordance with a well-established protocol. Manufacturer: Varies

2.3.2 *Demeclocycline (Declomycin)*

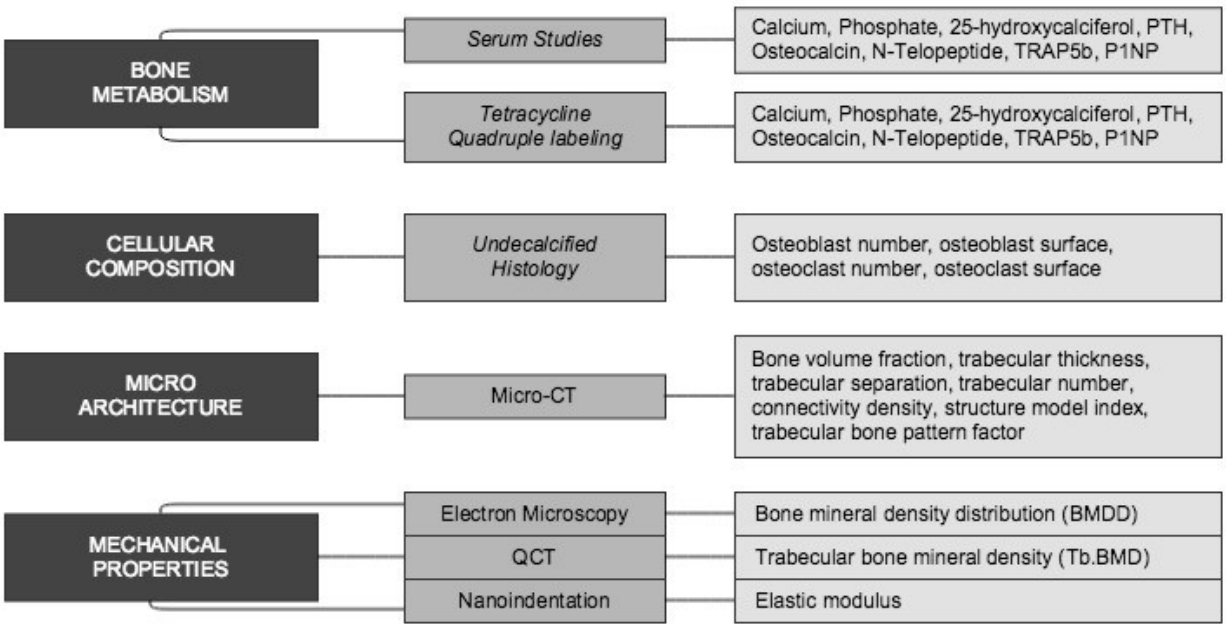
Demeclocycline is an FDA-approved anti-microbial drug, available in the US in generic form. It is a tetracycline derivative, and has a similar safety profile. All associated adverse effects are minimal and well-documented. Demeclocycline can be safely self-administered by the patient in a form of oral pill on outpatient basis. In this study, it will be used as an accessory fluorescent label (in addition to tetracycline) for dynamic histomorphometry studies, in accordance with a well-established protocol. Manufacturer: Varies

2.4 Correlative Studies Background

We hypothesized that bone degradation after irradiation is associated with detectable changes in serum markers, bone metabolism, bone structure and mechanical properties. In order to characterize these changes, we designed a series of *in vivo* and *in vitro* experiments, summarized in

Figure 4.

Figure 4: Experimental Design: Summary of the Proposed Experimental Techniques and Measurements to be Collected in the Study



Data Collection: We will characterize changes in the bone by evaluating the stucture and cellular composition of bone, bone metabolism and bone mechanical properties. This figure summarizes the experimens we plan tp perform and the data we plan to collect.

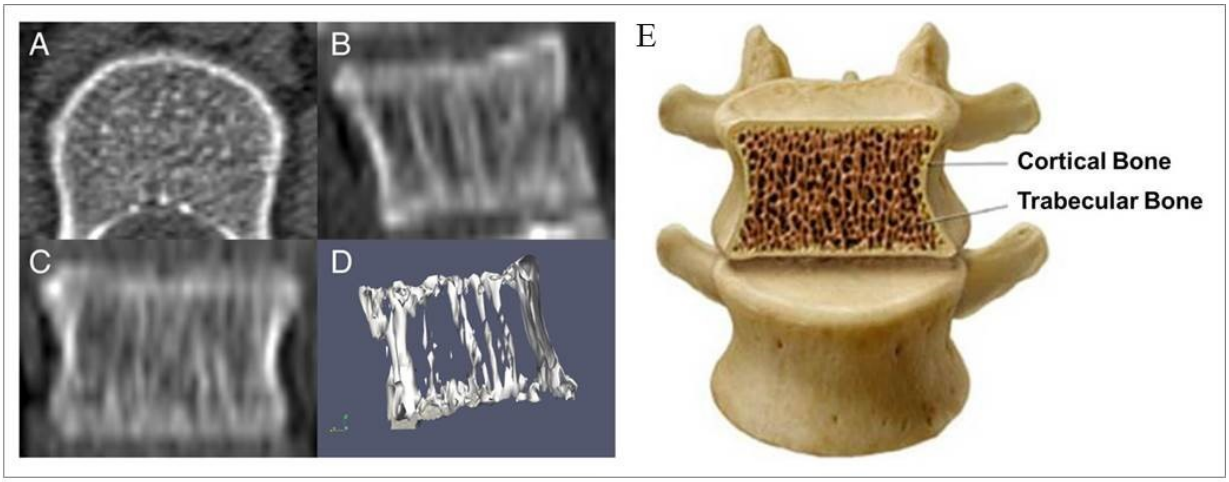
2.4.1 *Serum Markers*

A number of circulating serum molecules are used clinically as markers of bone turnover rate and bone remodeling. N-Telopeptide (NTX) is a breakdown products of Type I collagen molecule, which is particularly abundant in bone. It has been shown to be useful in predicting the risk of fractures [32] and other skeletal complications in cancer patients [17]. We will als test serum tartrate-resistant acid phosphatase 5b (TRAP5b). TRAP 5b may reflect defferent aspects of osteoclast function; osteoclast adhesion, osteoclast number and degradation of non-collagenous proteins [37, 38]. We will use these measurements as markers of *bone resorption*. In addition, we will measure several markers of *bone formation*: osteocalcin (OC) and type 1 procollagen N-terminal (P1NP).
In addition to bone-specific markers, we will measure calcium (as part of s atandard metabolic panel), phosphate, parathyroid hormone levels (PTH), and 25-hydroxycalciferol as indicators of systemic metabolism that can affect bone health. The changes in serum bone turnover markers are expect to predate fractures, if fractures do occur. While we expect the markers to increase with the onset of radiation, we will follow the trend after the radiation has b een discontinued to see if and when the markers begin to return to normal.

2.4.2 *QCT*

Quantitative computer tomography analysis (QCT) is a high resolution imaging technique that can detect changes in bone mineralization density and trabecular bone density [20, 33]. Figure 5 (E) shows the difference between two bone types. Cortical bone is thick and solid, and provides the weight-bearing strength of bone. Trabecular bone is porous, and has a woven-like structure. Radiation causes sclerosis of the trabecular bone, rendering it solid and dense, like cortical bone. Despite this increase in density, irradiated bone becomes brittle and prone to fractures. Traditional method of measuring bone density by dual-energy X-ray absorptiometry (DEXA) measures an average density of cortical and trabecular bone. It cannot differentiate between normal bone and fragile, irradiated bone affected by sclerosis. QCT can measure the density of trabecular bone directly. Figure 5(A-C) shows the axial, coronal and sagittal scans of the lumbar vertebral body by QCT. A three-dimensional reconstruction of a slice through the vertebral body allows determination of the architecture of the trabecular bone, as illustrated in Figure 5(D).

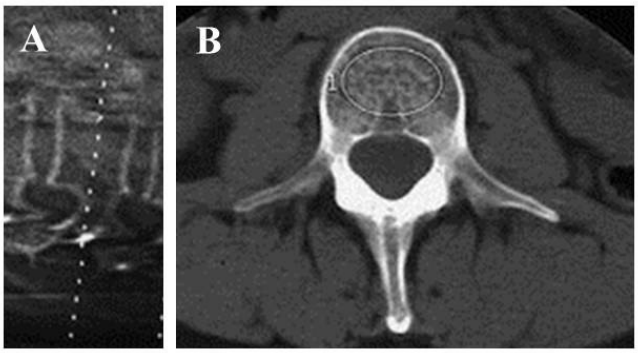
Figure 5: Determination of Bone Structure in the Adult Spine by Quantitative CT (QCT)



Calculation of the bone mineral density and trabecular bone density by QCT in the adult spine. A. Axial cut through the body of the L1 vertebra showing the difference in density of the cortical bone (bright white) and trabecular bone (gray). B. Coronal and C. Sagittal reconstructions of the same L1 vertebra show the woven porous structure of the trabecular bone. D. A rendered, sagittal slice of 10 mm thickness shows the trabecular structure in 3-D. E. Illustrates the difference in the structure of the cortical and the trabecular bone in the lumbar vertebral body.

Bone density can be calculated from the radiopacity of the bone expressed in Hounsfield units, averaged over a small region of interest (ROIs) as shown in Figure 6. In the lumborosacral spine, ROIs are drawn through the anterior aspect of the L4, the sacral alae, and midline of S1, avoiding any areas of abnormal anatomy or sclerosis due to fractures or degenerative changes. The Hounsfield unit values are then entered into software to calculate values for the trabecular bone mineral density (Tb. BMD) in the region of interest.

Figure 6: Definition of ROI in the Lumbar Spine



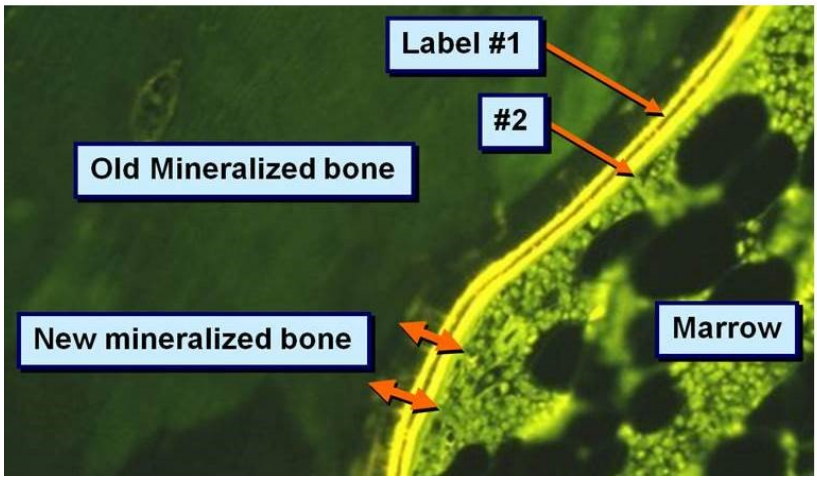
Determination of the Regions of Interest (ROI) in the Lumbar Spine. A. Shows the level of the axial cut through which the scan was taken. B. The elliptical area drawn around an area of the lumbar vertebral body is an ROI used to calculate the trabecular bone density.

2.4.3 *Bone Histomorphometry*

Histomorphometry is broadly defined as the measurement of the shape or form of a tissue. Bone histomorphometry is a quantitative analysis of bone structure and bone remodeling. It provides valuable information on the amount of bone and its cellular activity. *Static histomorphometry* allows quantitative evaluation of bone structure and cellular content at any particular time point. Undecalcified bone samples embedded in plastic resin are used for this purpose. Cellular content of is evaluated by immunohistochemical staining methods. Changes in bone structure and the volume of mineralized tissue are assessed by micro-computed tomography, quantitative back scatter electron imaging, and nanoindentation.

Dynamic histomorphometry provides a quantitative assessment of the extent of bone formation over a specific period of time (usually 10-14 days). If a fluorescent label, such as tetracycline, is present, it becomes incorporated into the newly formed bone, leaving a clear linear record of the site where mineralization was occurring (see Figure 7). If a second label is given 10-14 days after the first one, one can calculate the *mineral apposition rate* (MAR, mm/day) as the distance between the midpoints of the two consecutive labels, divided by the time interval between the labeling periods. The *bone formation rate* (BFR/BS, mm^3/mm^2/day) is the amount of newly formed bone seen under the fluorescent microscope divided by the time interval during which the label was given. We will use a quadruple labeling scheduele, using two sets of double tetracycline labels witch differ in color under fluorescent light. This way we will be able to distinguish the first and second label set. This technique has the great advantage that the patient can serve as his or her own pretreatment control, eliminating problems caused by the large intersite variablility in histomorphometric variables [39].

Figure 7: Visualization of Tetracycline Labeled Bone by Fluorescent Microscopy



Tetracycline Double Label. Older bone is bound to the first label and is located further away from the marrow side. Newly mineralized bone is closer to the marrow side, and is bound to the second label, which was administered later in the time course of the experiment.

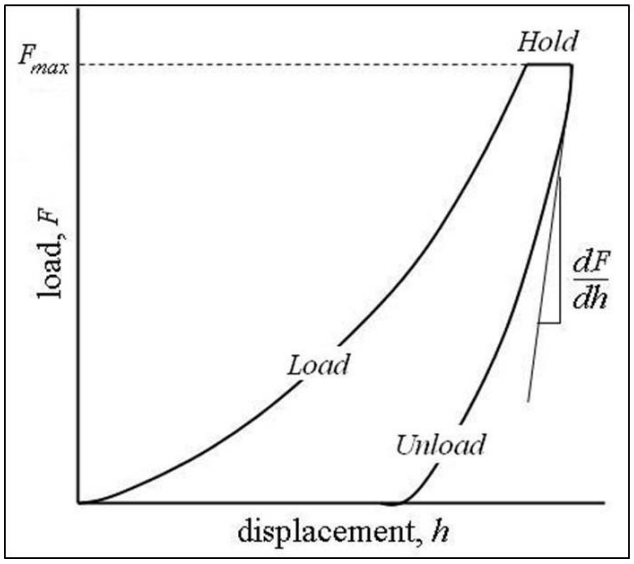
2.4.4 *Micro-computed tomography (micro-CT)*

Microtomography (micro-CT) is used for in vitro bone studies. It uses a similar technique to conventional X-ray tomography systems (regular CT scans), but with much finer resolution. Morphological parameters of any object can be analyzed with very high precision using three-dimensional reconstructions. The process is non-destructive and requires no special preparation of the specimen. Micro-CT can measure static morphological parameters of bone microscopic architecture. The bone volume fraction (BV/TV), trabecular thickness (Tb. Th.), trabecular separation (Tb. Sp.), trabecular number (Tb. N.), Connectivity density (Conn.D), structure model index (SMI), and trabecular bone pattern factor (TBPf) provide information about the characteristics of the trabecular bone structure. In networks of trabecular bone, connectivity density provides an estimation of the number of trabecular connections per unit volume. The structure model index determines the geometry of the intertrabecular network.

2.4.5 *Nanoindentation*

Nanoindentation is a powerful method for investigating the mechanical properties of materials in small dimensions. This is done by pressing a hard diamond tip into the sample of the material of interest with a known amount of force. Traditional indentation methods measured hardness of materials from the area of the “footprint” left in the sample by the diamond tip. Nanoindentation creates “footprints” in the submicrometer range, much too small to examine without a powerful microscope. Instead, it uses specialized depth sensing equipment to make tiny indentations in the surface. At the same time it records the load and displacement of the indenter with very high accuracy and precision. A load-displacement curve is constructed from the recorded values (Figure 8). Experimental data can be interpreted using analytical models to obtain hardness, the elastic modulus, and other mechanical properties.

Figure 8: Schematic Representation of a Typical Load-Displacement Curve



Load-Displacement Curve. A typical Load-Displacement curve is constructed from the load and the displacement of the indenter. It has three distinct portions labeled "load", "hold" and "unload". They refer to the corresponding stages of the experiment, during which the load on the indenter behaves differently. When the load is increased, the indenter sinks into the material due to both elastic and plastic deformation ("load"). If the load is held constant, the indenter continues to sink into the material due to time-dependent deformation or creep ("hold"). When the indenter is unloaded, the material recovers by a process that is primarily elastic ("unload").

We will measure the elastic modulus of bone from the unloading segment of the load-displacement curve using the Oliver-Pharr method. The elastic modulus is a measurement of bone's ability to resist mechanical stress. Healthy bone is strong and it effectively resists the outside forces exerted on it. When the body is in the upright position, gravity exerts a significant force on the bones of the spine and pelvis causing deformation. Healthy bone resists deformation: it returns to its original form when the force is removed. In contrast, bone weakened by osteoporosis or radiation cannot resist stress exerted by normal activities, like walking. Gravity causes it to deform permanently; even when the force is removed or reduced, the deformation remains. This is the mechanism proposed to explain the development of sacral insufficiency fractures after radiation. We want to measure the elastic modulus of bone in the sacrum before and after radiation to characterize the effect of radiation on the elastic properties. It is exceedingly likely that the pathogenesis of radiation-induced sacral insufficiency fractures is multifactorial. Given this complexity, the ability to isolate the effect of radiation on elastic property makes nanoindentation a uniquely valuable tool for our study.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

All laboratory tests that are a part of the eligibility criteria must be completed within 14 days prior to the date of registration. Diagnostic tests that are a part of the eligibility criteria must be performed within 30 days of the date of registration. Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Study participants must have histologically confirmed primary malignant bone tumor in the sacrum for which surgery and radiation or radiation alone are planned.

3.1.2 Age 18 years or older. In children under the age of 8, tetracycline derivatives have been reported to stain tooth enamel yellow color. These considerations lead us to exclude young persons under the age of 18 from the study.

3.1.3 Participants in the surgical arm must have normal organ and marrow function as defined below:

- Total bilirubin within normal institutional limits
- AST (SGOT)/ALT (SGPT) < 2.5 X institutional upper limit of normal
- Creatinine within normal institutional limits or creatinine clearance > 60 mL/min/1.73 m2 for subjects with creatinine levels about institutional normal limit

3.1.4 The effects of tetracyclines and radiation used in computer tomography on the developing human fetus are known to be detrimental. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and until after the last study related computer tomography scan . Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.5 Ability to understand and willingness to sign a written informed consent.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Participants who have had surgery, chemotherapy, or radiotherapy of the sacrum prior to entering the study

3.2.2 History of allergic reactions attributed to compounds of similar chemical or biologic composition to tetracyclines.

3.2.3 Pregnant or nursing

3.2.4 Uncontrolled inter current illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

We will not restrict subject selection by race, gender, or age group. The external validity of the results obtained in this study would benefit from population diversity. Since the study aims at improving outcomes for all patients with bone tumors, exclusion of any group that can be affected by the disease can result in the failure to collect clinically important information.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin participation in the protocol. Issues that would cause delays should be discussed with the Principal Investigator. If a participant does not participate in the protocol following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

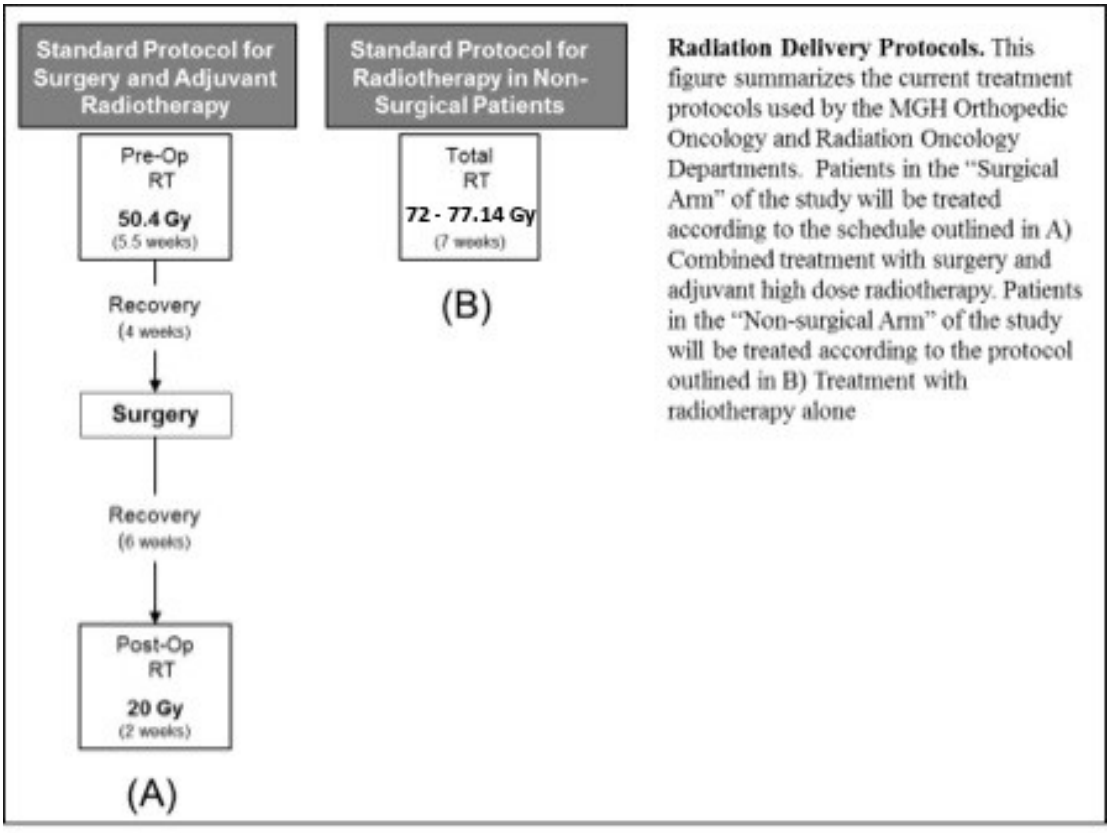
4. The QACT Registrar will (a) validate eligibility and (b) register the participant on the study.

5. The QACT Registrar will send an email confirmation of the registration to the person initiating the registration immediately following the registration.

5. TREATMENT PLAN

Study participants will receive treatment as determined by their treating physicians at the Massachusetts General Hospital for Malignant Bone Tumors of the Sacrum. This may be radiation alone or pre-operative radiation therapy followed by surgery. The treatment plan including MRI and CT imaging is determined by the clinical situation and not by the study. Patients planned for surgery typically receive pre-operative MRI of their pelvis and/or sacrum. In addition, patients often receive a pelvic or sacral computed tomographic image. A CT of the chest is performed to evaluate for pulmonary metastasis. Figure 9 outlines the current standard radiation treatment options. Study participants may also receive radiation therapy as part of another clinical trial. For surgical candidates, the standard treatment includes pre-operative radiation of 50.4 Gy, followed by a recovery period of approximately 4 to 5 weeks. Surgery involves removing the malignant tumor in the sacrum in one piece, preferably with a cuff of normal tissue around the tumor. After approximately 6 weeks of recovery, to allow the surgical incision to heal, the patient is treated with another 19.8 Gy up to 27 Gy of radiation postoperatively depending on the final margin status (higher for gross residual disease) , as outlined in Figure 9(A). If the wound is not healed or there is another medical reason to delay adjuvant radiation, then radiation may begin later. The patients are typically followed every 3 months with an MRI of the pelvis/sacrum to evaluate for a local recurrence. A CT scan is often ordered if there is a concern for a fracture. In addition, patients typically receive a chest x-ray at 3 months and 9 months after surgery. A chest CT is ordered at 6 months and 12 months after surgery. The same protocol is used during the second year of follow up. Non-surgical candidates receive 72 up to 77.4 Gy of radiation depending on the histology (72 Gy for osteosarcoma and chondrosarcoma and 77.4 Gy for chordoma) as outlined in Figure 9 (B).

Figure 9: Radiation Treatment Flowchart



5.1 Pre-treatment Criteria

Not applicable, as long as labs are within normal limits (see Section 3.1.3)

5.2 Bone Labeling Drug Administration

For participants in the non-surgical arm, no bone labeling drugs are administered. These participants will receive a standard radiation regimen with blood samples and imaging (QCT) only as their participation in this study. For participants in the surgical arm, the bone labeling drugs are described below.

5.2.1 *Demeclocycline*

Participants in the surgical arm will self-administer the first label set; demeclocycline 150 mg PO QID (daily dose 600 mg) during 2 periods of 3 days, starting day 18 and day 3 prior to the start of their schedueled pre-operative radiotherapy. Participants will be given a drug diary (Appendix 1) to keep a record of the dosing. Demeclocycline comes in a form of an oral pill and does not require any special equipment for administration. It has a well-established safety profile with minimal adverse effects. No observation period or vital signs monitoring is required. Demeclocycline should be administered 1 hour before or 2 hours after food or milk, with adequate amounts of fluid to decrease the risk of esophageal irritation and ulceration. If a dose is vomited, the participant should contact the research coordinator, so we can provide an extra dose. Contact information will be provided in the drug diary.

5.2.2 *Tetracycline*

Participants in the surgical arm will also self-administer the second label set; tetracycline 250mg PO QID (daily dose 1000 mg) during 2 periods of 3 days, starting day 24 and day 9 prior to their scheduled surgery. Participants will be given a drug diary (Appendix 1) to keep a record of the dosing. Tetracycline comes in a form of an oral capsule and does not require any special equipment for administration. It has a well-established safety profile with minimal adverse effects. No observation period or vital signs monitoring is required. Tetracycline should be administered on an empty stomach: 1 hour prior to meals, or 2 hours after meals. If a dose is vomited, the participant should contact the research coordinator, so we can provide an extra dose. Contact information will be provided in the drug diary.

5.3 Other Studies

As outlined in section 8.3.

5.4 Definition of Dose-Limiting Toxicity

N/A

5.5 General Concomitant Medication and Supportive Care Guidelines

Patients may receive all concomitant therapy deemed necessary to provide adequate support for any standard treatment or study related events.

5.6 Duration of Therapy

Standard treatment will continue for study participants as long as clinically indicated until the standard treatment is completed. As noted above, participants in the radiation arm will provide blood samples for up to 6 months after completion of radiation therapy. Participants in the surgical arm will self administer the bone labeling drugs as described above and will provide blood samples for up to 6 months after completing radiation therapy.

5.7 Duration of Follow Up

Participants will be followed for radiographic and/or clinical evidence of fracture and assessment of quality of life for 12 months after their surgery/radiotheraphy or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for Removal from Study

Participants will be removed from study if any of the adverse events listed in section 6.2.2 or 6.1.2 occur. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Dr. Schwab at 617-724-8636 or MGH pager #15724

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

If possible, symptoms will be managed symptomatically. In the case of toxicity, appropriate medical treatment will be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

All drugs in this study have been in routine clinical use for many years, and are associated with rare adverse effects. Additional radiation exposure is is less than the yearly natural background radiation in the US. A list of the adverse events and potential risks associated with the drugs administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 *Adverse Event List for Tetracycline*

Significant Adverse Reactions (Rare):

- Cardiovascular: Pericarditis
- Central nervous system: Bulging fontanels in infants, increased intracranial pressure, paresthesia, pseudotumor cerebri
- Dermatologic: Exfoliative dermatitis, photosensitivity, pigmentation of nails, pruritus
- Gastrointestinal: Abdominal cramps, anorexia, antibiotic-associated pseudomembranous colitis, diarrhea, discoloration of teeth and enamel hypoplasia (young children), esophagitis, nausea, pancreatitis, staphylococcal enterocolitis, vomiting
- Hematologic: Thrombophlebitis
- Hepatic: Hepatotoxicity
- Renal: Acute renal failure, azotemia, renal damage
- Miscellaneous: Anaphylaxis, candidal superinfection, hypersensitivity reactions, superinfection

Contraindications: Hypersensitivity to demeclocycline, tetracyclines, or any component of the formulation

Concerns related to adverse effects:

- Increased blood urea nitrogen (BUN): May be associated with increases in BUN secondary to antianabolic effects; use caution in patients with renal impairment.
- Photosensitivity: May cause photosensitivity; discontinue if skin erythema occurs. Use skin protection and avoid prolonged exposure to sunlight; do not use tanning equipment.
- Pseudotumor cerebri: Has been (rarely) reported with tetracycline use; usually resolves with discontinuation.
- Superinfection: Prolonged use may result in fungal or bacterial superinfection, including *C. difficile*-associated diarrhea (CDAD) and pseudomembranous colitis; CDAD has been observed >2 months postantibiotic treatment.

Disease-related concerns:

- Hepatic impairment: Use with caution in patients with hepatic impairment; dose adjustment and/or adjustment to interval frequency recommended. Hepatotoxicity and hepatic failure have been reported rarely with use.
- Renal impairment: Use with caution in patients with renal impairment; dosage adjustment recommended. Nephrotoxicity has also been reported with use, particularly in the setting of cirrhosis.

Special populations:

Pediatrics: May cause tissue hyperpigmentation, enamel hypoplasia, or permanent tooth discoloration; use of tetracyclines should be avoided during tooth development (children <8 years of age) unless other drugs are not likely to be effective or are contraindicated.

Pregnancy: Do not use during pregnancy or nursing. In addition to affecting tooth development, tetracycline use has been associated with retardation of skeletal development and reduced bone growth

6.1.2 *Adverse Event List for Demeclocycline*

Significant Adverse Reactions (Rare):

- Cardiovascular: Pericarditis
- Central nervous system: Bulging fontanels (infants), dizziness, headache, pseudotumor cerebri (adults)
- Dermatologic: Angioedema, anogenital inflammatory lesions (with monilial overgrowth), erythema multiforme, erythematous rash, exfoliative dermatitis (rare), maculopapular rash, photosensitivity, pigmentation of skin, Stevens-Johnson syndrome (rare), urticaria
- Endocrine & metabolic: Microscopic discoloration of thyroid gland (brown/black), nephrogenic diabetes insipidus, thyroid dysfunction (rare)
- Gastrointestinal: Anorexia, diarrhea, dysphagia, enterocolitis, esophageal ulcerations, glossitis, nausea, pancreatitis, vomiting
- Genitourinary: Balanitis
- Hematologic: Eosinophilia, neutropenia, hemolytic anemia, thrombocytopenia
- Hepatic: Hepatitis (rare), hepatotoxicity (rare), liver enzymes increased, liver failure (rare)
- Neuromuscular & skeletal: Myasthenic syndrome, polyarthralgia, tooth discoloration (children <8 years, rarely in adults)
- Ocular: Visual disturbances
- Otic: Tinnitus
- Renal: Acute renal failure, BUN increased
- Respiratory: Pulmonary infiltrates
- Miscellaneous: Anaphylaxis, anaphylactoid purpura, fixed drug eruptions (rare), lupus-like syndrome, superinfection, systemic lupus erythematosus exacerbation

Contraindications: Hypersensitivity to demeclocycline, tetracyclines, or any component of the formulation

Concerns related to adverse effects:

- Diabetes insipidus syndrome: Dose-dependent nephrogenic diabetes insipidus is common with use; however, this adverse event of demeclocycline has been used as a therapeutic advantage in the off-label use of hyponatremia associated with SIADH.
- Increased BUN: May be associated with increases in BUN secondary to antianabolic effects; use caution in patients with renal impairment.
- Photosensitivity: May cause photosensitivity; discontinue if skin erythema occurs. Use skin protection and avoid prolonged exposure to sunlight; do not use tanning equipment.
- Pseudotumor cerebri: Has been (rarely) reported with tetracycline use; usually resolves with discontinuation.
- Superinfection: Prolonged use may result in fungal or bacterial superinfection, including *C. difficile*-associated diarrhea (CDAD) and pseudomembranous colitis; CDAD has been observed >2 months postantibiotic treatment.

Disease-related concerns:

- Hepatic impairment: Use with caution in patients with hepatic impairment; dose adjustment and/or adjustment to interval frequency recommended. Hepatotoxicity and hepatic failure have been reported rarely with use.
- Renal impairment: Use with caution in patients with renal impairment; dosage adjustment recommended. Nephrotoxicity has also been reported with use, particularly in the setting of cirrhosis.

Special populations:

Pediatrics: May cause tissue hyperpigmentation, enamel hypoplasia, or permanent tooth discoloration; use of tetracyclines should be avoided during tooth development (children <8 years of age) unless other drugs are not likely to be effective or are contraindicated.

Pregnancy: Do not use during pregnancy. In addition to affecting tooth development, tetracycline use has been associated with retardation of skeletal development and reduced bone growth

6.1.3 ***Radiation Exposure Risks***

Will perform a volumetric duel energy CT of L1, L2, and S1 using a second-generation dual-source 128-row multi-detector CT scanner (Somatom De nition Flash; Siemens Medical Solutions, Forchheim, Germany). Subjects will be positioned on a quantitative CTPro calibration phantom (Mindways Software). Helical scans will be performed at 80 and 140 kV by using 210 and 80 mAs, respectively. Other scanning parameters included 1-second gantry rotation time, 0.9:1 pitch, and 64 3 0.6-mm detector configuration with double z-sampling. The images will be reconstructed at 2-mm section thickness and 2-mm section interval by using the I31f reconstruction kernel with a sinogram-affirmed iterative reconstruction (SAFIRE; Siemens Healthcare) setting of 2. The effective dose for each DECT scan is about 2 mSv. For 2 scans, the total effective dose is about 4 mSv which is about the annual background radiation dose.

6.2 **Toxicity Management**

6.2.1 ***Management of Tetracycline Toxicity***

Renal / Hepatic Impairement: Tetracycline will not be administered to patients with pre-existing renal or hepatic impairment.

Photosensitivity: Patients will be advised to use skin protection while taking tetracycline. In addition, patients will be advised against prolonged exposure to sunlight and use of tanning equipment. If skin erythema occurs, patients will be advised to discontinue taking the drug and contact the study team.

Superinfection: Patients will be informed of this risk, and will be closely monitored for any GI symptoms. Patients will be advised to discontinue taking the drug and contact their primary care provider and the study team, if any symptoms of infection occur.

Pseudotumor: Patients will be advised to discontinue taking the drug and contact the study team if headache, photophobia, gait disturbances, of any other neurological deficits occur.

Special populations: pregnant and lactating women and children will be excluded from the study due to the concerns related to tetracycline use in these populations (outlined in Section 6.1.1 Adverse Effects of Tetracycline 6.1.2).

6.2.2 *Management of Demeclocycline Toxicity*

Diabetes Insipidus: Patients will be advised to discontinue taking demeclocycline and contact their PCP and the study team, if they experience excessive thirst, urination, headache, dizziness, nausea/vomiting, or any other concerning symptoms.

Renal / Hepatic Impairement: Demeclocycline will not be administered to patients with pre-existing renal or hepatic impairment.

Photosensitivity: Patients will be advised to use skin protection while taking demeclocycline. In addition, patients will be advised against prolonged exposure to sunlight and use of tanning equipment. If skin erythema occurs, patients will be advised to discontinue taking the drug and contact the ,study team.

Superinfection: Patients will be informed of this risk, and will be closely monitored for any GI symptoms. Patients will be advised to discontinue taking the drug and contact their primary care provider and the PI study team, if any symptoms of infection occur.

Pseudotumor: Patients will be advised to discontinue taking the drug and contact the study team if headache, photophobia, gait disturbances, of any other neurological deficits occur.

Special populations: pregnant and lactating women and children will be excluded from the study due to the concerns related to tetracycline use in these populations (outlined in Section 6.1.2 Adverse Effects of Demeclocycline6.1.2).

6.2.3 *Management of Radiation Toxicity*

Additional radiation dose is approximately 4 mSv. This amount of radiation is less than the yearly natural background radiation in the US..We do not anticipate any toxic effects of this dose of radiation to the patients. However, patients will be advised that radiation is a potential teratogen. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

6.3 **Dose Modifications/Delays**

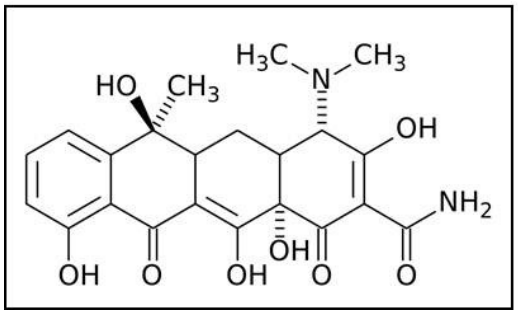
N/A

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Tetracycline

7.1.1 *Description*

The chemical name is: (4*S*,6*S*,12a*S*)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide. The molecular formula is $C_{22}H_{24}N_2O_8$The Molecular weight is 444.435 g/mol



Tetracycline

Bioavailability: 60–80%
Metabolism: Not Metabolized
Half-life: 6-11 hours
Excretion: Renal and Fecal

7.1.2 *Form*

Capsule, oral, as hydrochloride: 250 mg, 500 mg

7.1.3 *Storage and Stability*

All pharmaceuticals will be stored at the Massachusetts General Hospital Oncology Clinical Trials Pharmacy (Yawkey-7).

7.1.4 *Compatibility*

N/A

7.1.5 *Handling*

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the antimicrobial drug in a self-contained and protective environment.

7.1.6 *Availability*

All pharmaceuticals used in this study will be obtained from the in-patient pharmacy at MGH. Dispense of the bone labeling drugs will happen during the last day of the radiotherapy. This will be approximately 4 days prior to the start of the labeleling cycle. The total amount of pills for the labelset (24 tablets) will be devided over 2 bottles, each containing 12 pills. The bottles will be labeled ‘Period 1’ and ‘Period 2’, indicating one of the 3 day periods during wich the pills are self-administered.

7.1.7 *Preparation*

All pharmaceuticals will be prepared at the Massachusetts General Hospital Clinical Trials Pharmacy. The bone labeling drugs will be devided in 2 bottles with 12 tablets per bottle. One bottle will contain the exact amount of pills that will be self-administred during one of the 3 day period. There will be 2 of these 3 day periods.

7.1.8 *Administration*

Participants in the surgical arm will self-administer tetracycline 250mg PO QID (daily dose 1000 mg) during 2 periods of 3 days with a 12 day interval, i.e. days 24 ,23 and 22 and days 9, 8 and 7 prior to the 1st stage of their scheduled surgery. Tetracycline should be administered on an empty stomach (ie, 1 hour prior to, or 2 hours after meals) to increase total absorption. Patients will be asked not to take aluminum containing antacids two hours before and after the medication has been given as it can interfere with bone binding. Patients will be instructed to take the bone labeling drug no more than 3 consecutive days.

7.1.9 *Ordering*

All pharmaceuticals will be ordered through the Massachusetts General Hospital Clinical Trials Pharmacy. The designated pharmacist for this study is Lalit Joshi at 617-643-1812.

7.1.10 *Accountability*

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the bone labeling drugs (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form

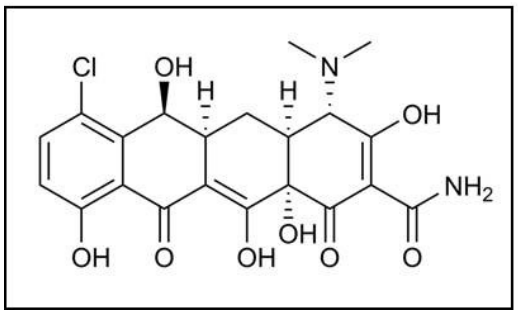
7.1.11 *Destruction and Return*

At the end of the study, unused supplies of tetracycline and demeclocycline will be disposed of according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 **Demeclocycline (Declomycin)**

7.2.1 *Description*

The chemical name is: 2*E*,4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-[amino(hydroxy)methylidene]-7-chloro-4-(dimethylamino)-6,10,11,12a-tetrahydroxy-1,2,3,4,4a,5,5a,6,12,12a-decahydrotetracene-1,3,12-trione. The molecular formula is$C_{21}H_{21}ClN_2O_8$ The Molecular weight is 464.853 g/mol



Demeclocycline

Bioavailability: 60–80%
Protein binding: 41–50%
Metabolism: Hepatic
Half-life: 10–17 hours
Excretion: Renal

7.2.2 *Form*

Tablet, oral, as hydrochloride: 150 mg, 300 mg

7.2.3 *Storage and Stability*

All pharmaceuticals will be stored at the Massachusetts General Hospital Oncology Clinical Trials Pharmacy (Yawkey-7).

7.2.4 *Compatibility*

N/A

7.2.5 *Handling*

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the antimicrobial drug in a self-contained and protective environment.

7.2.6 *Availability*

All pharmaceuticals used in this study will be obtained from the in-patient pharmacy at MGH. Dispense of the bone labeling drugs will happen during the last clinical visit before the start of the radiotherapy. This will be approximately 3-4 weeks prior to the start of the labeling cycle. The total amount of pills for the labelset (24 tablets) will be devided over 2 bottles, each containing 12 pills. The bottles will be labeled ‘Period 1’ and ‘Period 2’, indicating one of the 3 day periods during wich the pills are self-administered.

7.2.7 *Preparation*

All pharmaceuticals will be prepared at the Massachusetts General Hospital Clinical Trials Pharmacy. The bone labeling drugs will be devided in 2 bottles with 12 tablets per bottle. One bottle will contain the exact amount of pills that will be self-administred during one of the 3 day period. There will be 2 of these 3 day periods..

7.2.8 *Administration*

Participants in the surgical arm will self-administer demeclocycline 150 mg PO QID (daily dose 600 mg) during 2 periods of 3 days with a 12 day interval, i.e. day 18, 17 and 16 and day 3, 2 and 1 day prior to the start of their scheduled radiotheraphy. Demeclocycline will be administered 1 hour before or 2 hours after food or milk. Administer with adequate amounts of fluid to decrease the risk of esophageal irritation and ulceration. Patients will be instructed to take the bone labeling drugs no more than 3 consecutive days.

7.2.9 *Ordering*

All pharmaceuticals will be ordered through the Massachusetts General Hospital Clinical Trials Pharmacy. The designated pharmacist for this study is Lalit Joshi at 617-643-1812

7.2.10 *Accountability*

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the bone labeling drugs (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form.

7.2.11 *Destruction and Return*

At the end of the study, unused supplies of tetracycline and demeclocycline will be disposed of according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacokinetic Studies

N/A

8.2 Pharmacodynamic Studies

N/A

8.3 Special Studies

8.3.1 *Blood Draws*

We will follow the trends in serological markers over a 6 month period beginning prior to the first dose or radiation received by the patient. The study Schema outlines specific time points when we plan to draw

the blood samples. After all studies have been prefromed we will label the samples with a code. The key to the code will connect the sample with the subjects health information. The key to the code will be kept in a password protected computer of the prinicipal investigator. Samples will be stored at the BioBank and Research Laboratory Services fo the Maine Medical Center Research Institute.

Figure 10
What is measured:

- N-Telopeptide (NTX)
- Serum tartrate-resistant acid phosphatase 5b (TRAP5b)
- Osteocalcin (OC)
- Type 1 procollagen N-terminal (P1NP).
- PTH
- Calcium as part of a standard metabolic panel
- Phosphate

8.3.2 ***Quantitative CT Scans***

Patients in both arms of the study will undergo two QCT scans. First QCT will be done at Day 1, prior to initiation of radiation treatment. Patients in the Non-Surgical Arm will undergo a second QCT at six months after their first radiation treatment. In contrast, patients in the Surgical Arm will undergo their second QCT immediately prior to surgery, 10 weeks after the first scan. Once the spinal instrumentation is introduced during the surgery, it renders the analysis impossible due to artifact. Scans will be performed by the MGH Musculoskeletal Imaging Research Core (MIRC) under the direction of Dr. Martin Torriani (Radiology). We will use the QCT Pro protocol, detailed in Section 6.1.3 – Radiation Exposure Risks. Volumetric QCT of L1, L2 and S1 vertebrae will be obtained. Images will be saved in standard DICOM format and transferred to a PACS workstation for analysis.

What is measured:

- Trabecular bone mineral density (Tb. BMD)

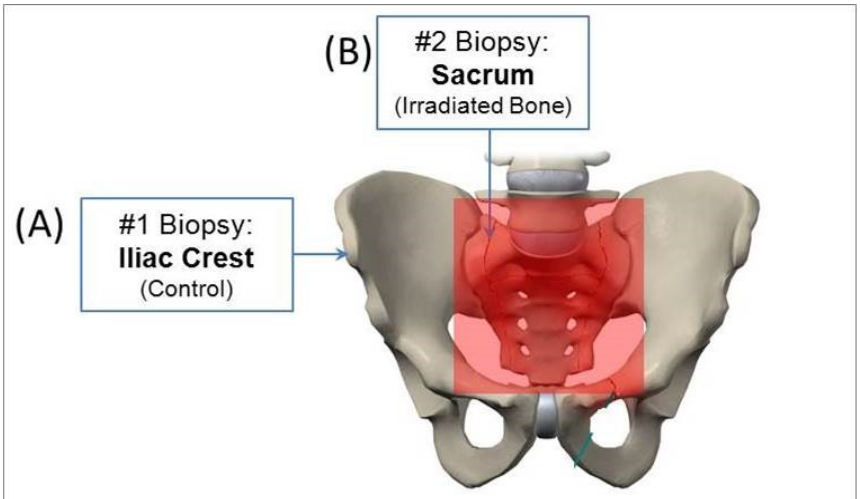
8.3.3 ***Bone Biopsies***

Bone biopsies will be performed on patients enrolled in the Surgical Arm of the study. Each patient will undergo two biopsies. At the time of each biopsy we plan to collect two bone samples. Figure 10 shows the location of the planned biopsy sites. The first biopsy (the control) will be taken from the iliac wing of the pelvis. This area lies outside of the radiated field, and will serve as a normal bone control. The second biopsy will be taken from the irradiated part of the sacrum. Bone biopsies will be evaluated for the end points of dynamic histomorphometry, and possible radiation-induced woven bone and bone mineralization abnormalities. Micro-CT will be performed on half of the bone samples prior to embedding to evaluate 3D trabecular microstructure. Biopsies that will be used for dynamic histomorphometry will be stored in 10% buffered formalin at room temperature in a dark space. We will send the biopsies to dr. Dempster at Colombia university for analysis. Tranfers will be done in batches using FedEx. After all studies have been performed we will label the biopsies with a code. The key to the code will connect the biopsy with the subjects health information. The key to the code will be kept a in password protected computer of the prinicipal investigator. Biopsies will be stored either at room

temperature (embedded specimen) or frozen (fresh specimen) at the Endocrine Unit Research Laboratory of the Massachusetts General Hospital.

Figure 10 shows the location of two biopsy sites.

Figure 10: Location of the Planned Bone Biopsy Sites.



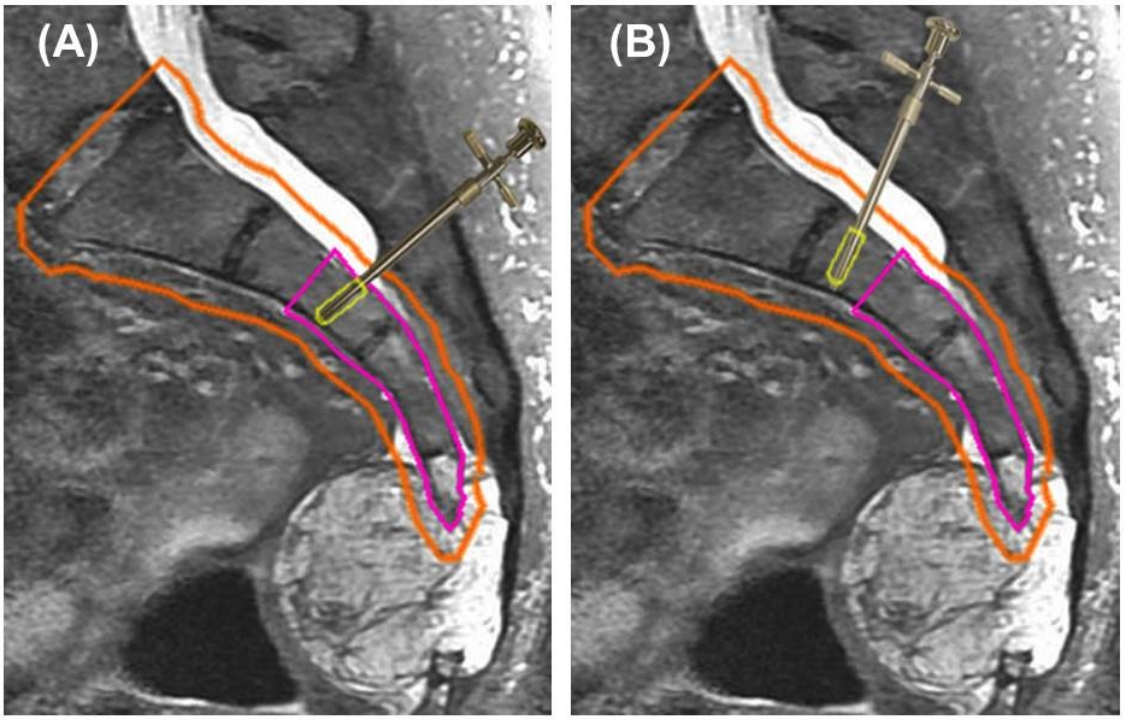
Location of the Biopsy Sites. A. Shows the approximate location of the anterior biopsy site at the anterior superior iliac spine. This area lies outside of the radiation field (indicated in red), and therefore will serve as normal bone controls. Irradiated bone samples will be collected from the sacrum. B. Shows the location of the sacral biopsy site. To maximize patient safety, we will try to remove the bone samples from the part of the sacrum removed during surgery. However, in some cases this may not be possible. In those patients we will collect bone from the area within 1 cm of the resection cut.

Biopsies will be done at the time of the scheduled tumor surgery. Sacral tumors are removed in stages. The first stage is done via anterior exposure, allowing access to the iliac crest. We will collect these samples first. Using a 7,5mm Rochester trephine two bone samples will be removed from the superior apex of the iliac wing in the midline (as defined as a point midway between the anterior superior and posterior superior iliac crests. In most cases, the biopsy site will be accessible through the incision made for the anterior approach. However, in those rare cases when the biopsy site is not accessible via the incision made as part of the standard of care, then a separate small incision (at most 3 cm long) will have to be made.

The second stage is done via posterior approach, providing access to the sacrum. We will collect the second biopsy samples during the second stage procedure. Using the same 7,5mm Rochester trephine, two bone samples will be removed from bone within one centimeter of the resection margin. To avoid unnecessary damage to healthy bone, we will attempt to collect the bone samples from the part of sacrum removed in surgery. After the tumor has been resected, it is normally brought to surgical pathology by the surgeon. There the specimen is examined by the pathologist. If there is enough normal bone remaining on the resected specimen, and the pathologist feels that using the normal bone will not interfere with his

ability to assess the margin of resection, then the biopsy will be obtained from the surgical specimen rather than from bone remaining on the sacrum still attached to the patient's spine. Figure 11 illustrates the two possible sites of the sacral biopsy.

Figure 11: Location of Sacral Biopsy Site



Sacral Biopsy. This sagittal MRI image of the sacrum shows a large tumor at the caudal end. The entire sacrum was irradiated in this patient; the extent of the radiation field is approximated by the orange outline. During surgery, an axial cut through the middle of the sacrum was made to remove the tumor with negative margins. The portion of the sacrum removed is outlined in purple. For the purposes of our study, we would always try to take the bone samples from the portion of the sacrum to be discarded (A). In those cases when the fragment removed doesn't have enough healthy tissue for a biopsy, the samples will be collected from the portion of the sacrum that remains attached to the patient's spine (B). The approximate location of the biopsy site is marked with a trephine in A and B.

8.3.4 *Undecalcified Histology*

One control and one experimental sample from the biopsies will be embedded in plastic resin (MMA/dibutylpthalate) then split in half using a fine handsaw (0.3mm kerf). So as to not alter the mechanical properties of bone, one half will be scanned by micro-CT then processed for thin section histology. The other half will be sectioned at 1 mm intervals for quantitative back scatter electron imaging followed by nano indentation.

8.3.5 *Immunohistochemistry*

Cellular content of bone can be evaluated by immunohistochemical staining methods. Osteoblasts will be counted on toluidine blue stained slides as blue/grey cuboidal cells in clusters at the bone surface. Osteoclasts will be counted on slides assayed for TRAP activity as TRAP-positive multinucleated cells.

What is measured:

- Osteoblast number (N.Ob/BPm, mm^{-1})
- Osteoblast surface (Ob. S/BS, %)
- Osteoclast number (N.Oc/BPm, mm^{-1})
- Osteoclast surface (Oc.S/BS, %)

8.3.6 ***Micro-CT***

Undecalcified biopsies will be scanned with a micro-CT system (X-Tek HMXST 225 microfocus X-ray system, Nikon Metrology, Tring,UK).

What is measured:

- Bone volume fraction (BV/TV)
- Trabecular thickness (Tb.Th.)
- Trabecular separation (Tb.Sp.)
- Trabecular number (Tb.N.)
- Connectivity density (Conn.D)
- Structure model index (SMI)
- Trabecular bone pattern factor (TBPf)

8.3.7 ***Nanoindentation***

Experimental design, data collection and analysis for nanoindentation will be done by our collaborator Dr. Mary Bouxsein, who has the expert knowledge and experience with this technology. Bone samples for nanoindendation cannot be embedded in paraffin because it would alter the elastic properties. The portion of the biopsy specimen for nanoindentation will be collected and stored in a small amount of normal saline solution at 4°C. Bone can remain stored in this fashion for several weeks without compromising the quality of the data. Measurements will be done with a nanohardness tester (Agilent G200 Nanoindenter, Santa ClaraCA) equipped with a diamond tip of Berkovich. The indentation will be performed with a load of 5 mN.

What is measured:

- Elastic modulus

8.3.8 ***Tetracycline Quadruple Labeling***

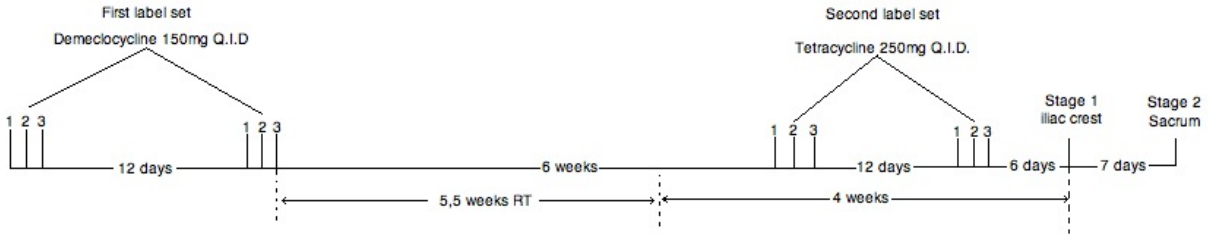
We will use a quadruple labeling scheduele, using two sets of double tetracycline labels witch differ in color under fluorescent light (ie tetracycline and demeclocycline). Figure 12 shows the schedule for the administration of the bone labeling drug. This way we will be able to distinguish the first and second label

set. This technique has the great advantage that the patient can serve as his or her own pretreatment control, eliminating problems caused by the large intersite variablility in histomorphometric variables

What is measured:

- Mineral apposition rate
- Mineral formation rate
- Mineralization lag time

Figure 12: Tetracycline Administration Schedule



9. STUDY CALENDAR

9.1.1 *Non- Surgical Arm Study Calendar*

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Final visit
Time point	Screening	Start RT Baseline	Halfway RT ~3.5 Weeks	End of RT ~7 Weeks	~3Months after RT	~6 Months after RT
Medical History	X					
Study Blood test		X	X	X	X	X
CT scan		X			X	
Pregnancy Test	X					

RT = radiotherapy
* Study visit are dependent on treatment events like start/end of pre- or post-op radiotherapy and surgery. Therefore treatment delay will result in delay of study visits.

9.1.2 *Surgical Arm Study Calendar*

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Final Visit
	Before Surgery				Surgery Week		After post-op RT	Follow-Up	
	Screening	Start RT Baseline	End RT ~5.5 Weeks	3-5 days before surgery	Surgery stage 1	Surgery stage 2	End post-op RT	1 month after post-op RT	4 months after post-op RT
Medical History	X								
Eligibility blood test	X								
CT scan		X		X					
Study Blood test		X	X				X	X	X
Bone Biopsy					X	X			
Dispense drugs	X		X						
Pregnancy Test	X								

RT = radiotherapy

* Cycle 1 drugs will be taken between visit 1 and 2. Cycle 2 drugs will be taken between visit 3 and 4* Study visit are dependent on treatment events like start/end of pre- or post-op radiotherapy and surgery. Therefore treatment delay will result in delay of study visits.

10. MEASUREMENT OF EFFECT

N/A for this corellative study

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 *Adverse Event (AE)*

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting any study procedure specified in the protocol.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 *Serious adverse event (SAE)*

A serious adverse event (SAE) is any adverse event, occurring as a result of one of the study procedures:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more sever form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 *Expectedness*

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 ***Expected adverse event***

Expected adverse events are those that have been previously identified as resulting from administration of the bone labeling drug. For the purposes of this

study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the bone labeling drugs.

11.1.3.2 ***Unexpected adverse event***

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 *Attribution*

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.
A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 *Serious Adverse Event Reporting*

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Joseph Schwab, M.D.
Office phone: 617-643-2483
Email: jhschwab@partners.org
Fax: 617-726-7587

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 *Non-Serious Adverse Event Reporting*

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

Joseph Schwab, M.D.
Office phone: 617-643-2483
Email: jhschwab@partners.org
Fax: 617-726-7587

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

11.6 Reporting to the Food and Drug Administration (FDA) N/A

11.7 Reporting to the NIH Office of Biotechnology Activities (OBA) N/A

11.8 Reporting to the Institutional Biosafety Committee (IBC) N/A

11.9 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.10 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 *Method*

The QACT will collect, manage, and monitor data for this study.

12.1.2 *Data Submission*

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 30 days of the last radiation treatment
Toxicity Report Form	Weekly during radiation treatment; Within 30 days of protocol defined follow up
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial as needed. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet as required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 **Monitoring**

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the Principal Investigator. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 **Protocol Review and Amendments**

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.
Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 **Informed Consent**

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the

consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidancewww.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf
- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 11 – Electronic Records; Electronic Signatures www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
    - Title 21 Part 56 – Institutional Review Boards www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
    - Title 21 Part 312 – Investigational New Drug Application www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- DF/HCC research policies and procedures http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-center Guidelines

N/A

13.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

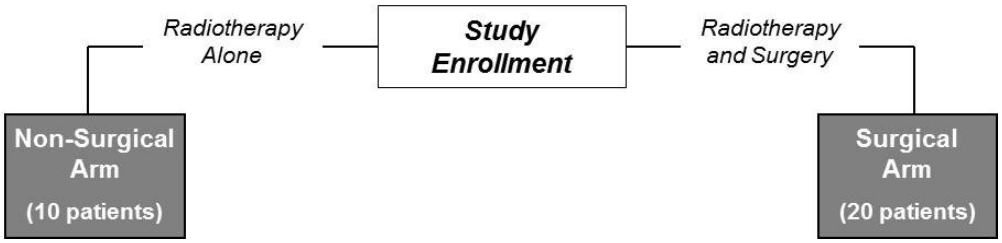
N/A

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

We will have two groups of patients, those treated with radiation alone, and combination of surgery and radiation. Figure 13 summarizes the proposed enrollment groups for this study, depending on the type of treatment patients are undergoing.

Figure 13: Proposed Subject Enrollment Plan



14.1.1 *Primary Endpoint*

- *Outcome of interest*: change in trabecular bone mineral density (Tb. BMD) measured by QCT of the lumbar spine at the start and at the end of radiation treatment

14.1.2 *Exploratory Endpoints*

- Longitudinal measurement of serum markers of bone turnover rate over 6 months
- Measurement of static and dynamic bone parameters summarized in Table 1.

Table 1: Exploratory Endpoints of the Study

Technique	Measurement
Immunohistochemistry:	Osteoblast number and surface Osteoclast number and surface

Micro-CT :	Bone volume fraction Trabecular number Trabecular thickness and separation Connectivity density Structure model index Trabecular bone pattern factor
Nanoindentation :	Elastic modulus
Tetracycline quadruple labeling :	Mineralization lag time Mineral apposition rate Mineral formation rate

14.2 **Sample Size/Accrual Rate**

Berthold and Haras published a range of normal reference values for the trabecular bone mineral density measured by quantitative CT of the lumbar spine in young adults. In healthy males and females the mean trabecular bone mineral density was 150mg /mL with a standard deviation of 20mg/mL at the start of puberty. In contrast, we expect our patients to be considerably older, to have one or many systemic illnesses, and to have tumors in the sacrum that can compromise bone quality. We expect that mean bone density at the start of the treatment in our population will be considerably lower. Assuming that it's at least 1 standard deviation less that of a normal young adult, we get an estimate of 130mg/dL at the start of treatment in all study patients. It is likely that our population will exhibit significant differences by sex owing to bone density being more affected by age in females than in males. In absence of prior studies, it is very difficult to estimate this difference. In fact, the purpose of this pilot study is in part to provide some numerical data to help guide future studies. At present, we must carry our sample size calculations keeping in mind these potential pitfalls.

The "event of interest" in this study is a change in trabecular bone density. Our hypothesis is that radiation induces loss of trabecular bone density in surgical and non-surgical patients. We would like to be able to detect a change in the average bone density of 10 mg/mL (approximately 5%) before and after radiation treatment. Assuming the standard deviation of the change we hope to observe is 15mg/dL, we get the following estimates.

Size = 30, Power = 0.942, alpha = 0.05
Size = 20, Power = 0.807, alpha = 0.05
Size = 10, Power = 0.469, alpha = 0.05

The main treatment arm in our patient population is the surgical patients. We plan to enroll 20 surgical patients given the results of the power analysis. The purpose of the non-surgical arm is to serve as a control for any confounding effects due to surgery. We plan to enroll 10 non-surgical patients. Smaller sample size significantly reduces the power of our analysis in the non-surgical arm. However, given that the vast majority of our patients receive surgery, the time it would take to enroll 20 patients into the non-surgical arm would undermine the purpose of doing an exploratory study to begin with.

The Kolmogorov–Smirnov test on the normality of the distribution of bone mineral density and trabecular bone mineral density values will be carried out in each group. To evaluate the effects of radiation, we will

use the Student t-test and Mann–Whitney test to compare the results from the iliac crest and the sacrum. We will use the Cox stratified model to identify confounding factors.

We plan to enroll 1-2 subjects per month. Subject enrollment is limited by patient availability.

The estimated study accrual by gender, race and ethnicity is shown in the table below.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	0	=	1
Not Hispanic or Latino	14	+	15	=	29
Ethnic Category: Total of all subjects	15	+	15	=	30
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	0	+	0	=	0
Black or African American	1	+	1	=	2
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	14	+	14	=	28
Racial Category: Total of all subjects	15	+	15	=	30

14.3 Stratification Factors

Patients will be stratified by the type of treatment they are undergoing (surgical vs. non-surgical).

14.4 Analysis of Secondary Endpoints

Paired t-test and Wilcoxon signed-rank test will be performed on the differences between the two groups in the average values of PROMIS T scores. A P-value of 0.05 will be considered to be statistically significant. Spearman and Kendall rank correlations will be used to measure the strength of dependence between the bone mineral density and exploratory variables whenever possible.

14.5 Reporting and Exclusions

14.5.1 *Evaluation of toxicity*

N/A

14.5.2 *Evaluation of response*

N/A

15. PUBLICATION PLAN

The results of the study will be made public within 24 months of the end of data collection. Our group plans to publish a report in a peer-reviewed journal. As an initial release, we plan to publish and present an abstract that meets the requirements of the International Committee of Medical Journal Editors at the Annual Meeting of the Sacral Tumor Research Group. A full report of the outcomes will be made public no later than three (3) years after the end of data collection.

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17. APPENDICES

17.1 Appendix 1: List of Figures

17.2 Appendix 2: Patient Drug Schedule And Diary

Study Drug Schedule

CYCLE 1

	Days before start radiotheraphy	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Start radiotheraphy
	Calendar Date	/	/	/													/	/	/	/
Time of Day to take Drug	Morning	Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)													Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)	
	Noon	Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)													Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)	
	Late afternoon	Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)													Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)	
	Night	Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)													Demeclocycline (150mg)	Demeclocycline (150mg)	Demeclocycline (150mg)	

STUDY DRUG INSTRUCTIONS:

Study Drug: Demeclocycline
How Much: Your dose is **150 mg**.
How Often: You will take each dose 4 times per day for 3 days.
When: You should take your dose 1 hour before or 2 hours after food or milk. You should take the dose with an adequate amount of fluids to reduce throat irritation. If a dose is vomited, please contact the research coordinator.

Contact information Research coordinator:
Olivier van Wulfften Palthe
e-mail: odvanwulfftenpalthe@mgh.harvard.edu
tel: +1 (857) 206-4112

Study Drug Schedule

CYCLE 2

	Days before FIRST Surgery	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	First Surgery
	Calendar Date	/	/	/													/	/	/							/
Time of Day to take Drug	Morning	Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)													Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)							
	Noon	Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)													Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)							
	Late afternoon	Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)													Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)							
	Night	Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)													Tetracycline (250mg)	Tetracycline (250mg)	Tetracycline (250mg)							

STUDY DRUG INSTRUCTIONS:

Study Drug: Tetracycline
How Much: Your dose is **250 mg**.
How Often: You will take each dose 4 times per day for 3 days.
When: You should take your dose on an empty stomach and either 1 hour before meals or 2 hours after meals. Please do not take aluminum containing antacids 2 hours before or after taking the dose. If a dose is vomited, please contact the research coordinator.

Contact information Research coordinator:
Olivier van Wulfften Palthe
e-mail: odvanwulfftenpalthe@mgh.harvard.edu
tel: +1 (857) 206-4112

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09 - 6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials _____ Date _____

FOR STUDY TEAM USE ONLY

Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

DOSING LOG

Study Drug Cycle 1: Demeclocycline 150mg

Please indicate the date, amount taken and any comments.

Days before START OF RADIO-THERAPH	Date - time	Amount Taken Drug Cycle 2	Comments
Ex:	6/1/2009 - 07:00	2	vomited hour later
Day 18			
Day 18			
Day 18			
Day 18			
Day 17			
Day 17			
Day 17			
Day 17			
Day 16			
Day 16			
Day 16			
Day 16			
Day 3			
Day 3			
Day 3			
Day 3			
Day 2			
Day 2			
Day 2			
Day 2			
Day 1			
Day 1			
Day 1			
Day 1			

Study Participant
Self-Administration
Study Drug Diary Cycle 1
Dana-Farber/Harvard Cancer Center

Participant Identifier: _____
Protocol #: 14-208
Your MD _____ Phone _____
Your RN _____ Phone _____

STUDY DRUG INSTRUCTIONS:

Study Drug Cycle 1: Demeclocycline

How Much: Your dose is 150 mg
How Often: You will take each dose 4 times per day for 3 days.
When: You should take your dose 1 hour before or 2 hours after food or milk.

SPECIAL INSTRUCTIONS:

Please take your dose 1 hour before or 2 hours after food or milk.
Please take the drug with a glass of water
Please do not take the drugs for more then 3 consecutive days
Please bring any unused study drug, all empty containers, and diary to the next clinic visit.
If a dose is vomited, please contact the research coordinator.

Contact information Research coordinator:
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e-mail: odvanwulfftenpalthe@mgh.harvard.edu
tel: +1 (857) 206-4112

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09 - 6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials _____ Date _____

FOR STUDY TEAM USE ONLY

Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

Study Participant
Self-Administration
Study Drug Diary Cycle 2
Dana-Farber/Harvard Cancer Center

Participant Identifier: _____
Protocol #: 14-208
Your MD _____ Phone _____
Your RN _____ Phone _____

STUDY DRUG INSTRUCTIONS:

Study Drug Cycle 2: Tetracycline

How Much: Your dose is 250 mg.
How Often: You will take each dose 4 times per day for 3 days.
When: You should take your dose on an empty stomach and either 1 hour before meals or 2 hours after meals.

SPECIAL INSTRUCTIONS:

Please take the drug on an empty stomach. This means 1 hour before or 2 hours after a meal
Please do not take aluminiun containing antiacids 2 hours before and after taking the drug
Please do not take the drugs for more then 3 consecutive days
Please bring any unused study drug, all empty containers, and diary to the next clinic visit.
If a dose is vomited, please contact the research coordinator.

Contact information Research coordinator:
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e-mail: odvanwulfftenpalthe@mgh.harvard.edu
tel: +1 (857) 206-4112

DOSING LOG

Study Drug Cycle 2: Tetracycline 250mg

Please indicate the date, amount taken and any comments.

Days before FIRST SURGERY	Date - time	Amount Taken Drug Cycle 2	Comments
Ex:	6/1/2009 - 07:00	2	vomited hour later
Day 24			
Day 24			
Day 24			
Day 24			
Day 23			
Day 23			
Day 23			
Day 23			
Day 22			
Day 22			
Day 22			
Day 22			
Day 9			
Day 9			
Day 9			
Day 9			
Day 8			
Day 8			
Day 8			
Day 8			
Day 7			
Day 7			
Day 7			
Day 7			

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity

17.3 Appendix 3: Summary of Experimental Procedures

In vivo studies (serum labs and imaging)

Experiment	Measurement
QCT	Trabecular bone mineral density (Tb.BMD)
Serum markers	N-Telopeptide (NTX) Tartrate-resistant acid phosphatase (TRAP) 5b Osteocalcin (OC) Total procollagen type 1 N-terminal propeptide (P1NP) PTH Ca (as part of a standard metabolic panel)

In vitro studies (on bone biopsy samples)

Experiment	Measurement
Immunohistochemistry	Osteoblast number (N.Ob/BPm, mm^{-1}) Osteoblast surface (Ob. S/BS, %) Osteoclast number (N.Oc/BPm, mm^{-1}) Osteoclast surface (Oc.S/BS, %)
Micro-CT	Bone volume fraction (BV/TV) Trabecular thickness (Tb.Th.) Trabecular separation (Tb.Sp.) Trabecular number (Tb.N.) Connectivity density (Conn.D) Structure model index (SMI) Trabecular bone pattern factor (TBPf)
Quantitative back scatter electron imaging (qBEI)	Bone mineral density (BMD)
Nanoindentation	Elastic modulus of bone
Tetracycline quadruple labeling	Mineral apposition rate Mineral formation rate Mineralization lag time