1. BACKGROUND AND RATIONALE

1.1 Cystic Fibrosis Related Bone Disease

Over the past several decades, life expectancy for patients with CF has increased significantly, from an average of 10 years of age in 1962 to more than 37 years of age in 2009. As patients live longer, other co-morbidities related to CF have become more prevalent, including CF-related bone disease.[1] The pathogenesis of a low BMD and increased fracture risk in patients with CF is currently not well understood.

Multiple clinical studies have reported that BMD measured by DXA is low in patients with CF.[2-5] For example, in a recent meta-analysis, the prevalence of T-scores between -1 and -2.5 in patients aged 18-32 years old with CF was 38%, and the prevalence of T-scores less than -2.5 was 23.5%, whereas the expected prevalence would be approximately 18% and 2.5% for people of this age.[6] In addition, bone turnover may be uncoupled in patients with CF with elevated bone resorption and low bone formation.[7,8]

Fracture rates in patients with CF are higher than in the healthy population.[9-12] In the previously mentioned meta-analysis, the prevalence of vertebral and non-vertebral fractures in adults with CF was 14% and 19.7%, respectively.[6] One study found that in 70 patients covering 1410 patient-years of analysis, fracture rates were twice as high in adolescents and young adults with CF compared to the general population.[9] In addition, kyphosis is also commonly seen in adolescents and young adults with CF.[11,13,14] Fractures and kyphosis in patients with CF can have severe repercussions. For example, rib and vertebral fractures can lead to pain, chest wall deformities, reduced lung volumes, ineffective cough and airway clearance, and ultimately compromised lung function. In addition, severe bone disease may obviate lung transplantation. Due to this high morbidity, CF related bone disease can significantly affect the health, well-being, and longevity of these patients.

CF patients have multiple risk factors for low bone density, including vitamin D deficiency, malabsorption, physical inactivity, malnutrition with low body weight, glucocorticoid use, diabetes, hormonal deficiencies, delayed puberty, inflammation, and failure to achieve peak bone mass. [1,2,7,15-17] In addition to these acquired factors, recent studies have also suggested that the CF genetic mutation in itself may play a causative role in low bone density and fracture seen in this population.

1.2 Potential Role of CFTR in CF Related Bone Disease

Cystic fibrosis is caused by a mutation in the *cftr* gene, which encodes the CF transmembrane conductance regulator (CFTR). CFTR traditionally serves as a chloride channel in epithelial cells and plays an important role in maintaining hydration required for mucillary clearance in pulmonary and intestinal tissue. Interestingly, recent evidence in mouse models suggests that CFTR is also present in bone cells and may play a role in bone formation and resorption. Knockout mice for *cftr (cftr-/cftr-)* have significantly decreased bone density and decreased bone formation rates compared to normal mice, even in the setting of normal lung and gastrointestinal function.[18-20] In addition, human

osteoblasts in culture have been shown to express CFTR.[21] Early data suggest that inhibiting CFTR expression in human osteoblasts may decrease osteoproteregin and prostaglandin E2, two important signaling molecules involved in bone formation and resorption. [22] This potential role of CFTR in bone resorption and formation is consistent with clinical studies in humans that have shown abnormalities in bone turnover markers and bone histomorphometric studies that reflect a decrease in bone formation and an increase in bone resorption.[8,23]

1.3 VX-770

VX-770 is a new medication that pharmacologically potentiates CFTR activity in patients with at least one copy of the G551D-CFTR mutation. Targeting CFTR systemically, VX-770 not only improved pulmonary function in these patients during phase 2 and 3 clinical trials, it also resulted in extrapulmonary improvements.[24,25] VX-770 was FDA approved in January 2012.

Given the possible role of CFTR in bone cells, VX-770 may also have a direct benefit on bone density and strength, but this has not yet been studied. This medication offers a unique opportunity to study the role of CFTR in human bone by assessing effects of pharmacologically increasing CFTR activity in subjects with G551D-CFTR mutations on bone micro-architecture and bone turnover markers. Because the effects of VX-770 on bone are of unknown magnitude, new imaging modalities like HR-pQCT that are highly reproducible and capable of detecting small changes are needed to be capable of detecting changes in bone that might otherwise be missed with standard imaging techniques such as DXA and pQCT.

1.4 Limitations of DXA and Standard pQCT

Several modalities are currently available for the clinical measurement of bone density, including dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Each of these modalities possess inherent limitations in the evaluation of CF-related bone disease due to unique factors complicating their interpretation.

At the present time, DXA is the primary method for measuring BMD and assessing fracture risk in both children and adults. DXA expresses bone mineral content (in grams) per unit area (cm^2) so that results are expressed as g/cm^2. Because DXA values are expressed per unit area rather than per unit volume, DXA does not truly give a measure of bone mineral density (which would be expressed as g/cm^3); rather, it gives values for "areal" or two-dimensional bone density. As such, DXA values are influenced by bone size, overestimating BMD for large bones and underestimating BMD for small bones, as are seen in many patients with CF. This artifact does not occur with techniques that provide volumetric (i.e., three-dimensional) measurements of BMD. Moreover, DXA cannot distinguish between trabecular and cortical bone.[26,27] Most importantly, some data suggest that DXA may not predict fracture risk accurately in patients with CF, such that they have increased risk of fracture even with normal DXA BMD measurements.[10]

pQCT is a modality that has been used as a method of evaluating BMD and some aspects of bone structure. Unlike DXA, pQCT assesses true volumetric bone density and it may be able to distinguish cortical and trabecular bone compartments. In adolescents and young adults with CF, one study using pQCT reported that cortical thickness of the radius was reduced in both men and women.[28] However, the radiation exposure from a QCT exam is about 100 fold higher than DXA, limiting its utility in children, particularly if serial measurements are planned. Additionally, the resolution of pQCT is 0.4 mm, which is not ideal for assessing small differences in cortical thickness and does not allow for assessment of other bone micro-architecture features including cortical porosity.

1.5 High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT)

HR-pQCT is a new imaging modality that has a resolution (voxel size) of 82 um, which allows for the assessment of the microstructure of bone (e.g., cortical thickness, trabecular number and thickness, trabecular spacing, and cortical porosity) in three dimensions and has the potential to provide a more accurate estimation of bone strength using microfinite element analyses. In addition, it measures true volumetric bone density, rather than areal bone density, of the total bone segment as well as the individual cortical and trabecular compartments. [29-31] HR-pQCT is highly reproducible and appears to give unique information that adds to the information provided by DXA. Furthermore, HR-pQCT is not limited by projection artifacts caused by bone size and is not affected by the amount or distribution of adipose tissue as can occur with DXA measurements. In addition, HR-pQCT also offers the advantage over standard pQCT of smaller voxel size (82um vs. 0.4mm), lower radiation dose, and faster scanning time. HR-pQCT is currently being studied as an alternative method of measuring bone density, predicting fracture, and assessing response to medications used to treat osteoporosis. Presently, studies using HR-pQCT in various disease populations, including type 2 diabetes and chronic kidney disease, have found significant differences in bone micro-architecture that were not captured by DXA BMD measurements.[32-36] Because radiation exposure is very low, HR-pQCT can be used for longitudinal studies to assess changes in volumetric bone density and bone micro-architecture over time.[37-39]

1.6 Current Proposal

We propose a prospective multiple cohort study with the goal of evaluating the skeletal effects of improving CFTR function in humans with CF. Subjects with at least one copy of the G551D mutation starting VX-770 will be evaluated using HR-pQCT at baseline and 12 and 24 months after initiating this medication and then compared to CF patients not receiving this medication as well as healthy subjects matched for age and gender. This study is highly innovative. Using cutting edge imaging techniques, we will assess the use of bone micro-architectural changes as a novel outcome in the assessment of medical interventions in CF patients. In addition, this study will provide important information regarding the pathogenesis of cystic fibrosis related bone disease and the underlying role of CFTR, and will help guide future management and prevention of this disease. These data can also serve as supportive evidence for future studies assessing the effects of VX-770, as well as to validate another outcome measure studying future CFTR modulators.

1.7 Preliminary Studies

To learn more about the underlying alterations in bone micro-architecture in CF patients, we are currently recruiting subjects with CF for a cross sectional study at MGH (protocol 2011P000893, SPID 978). The goal of this study is to use HR-pQCT to describe the micro-architectural basis for the differences in BMD in patients with cystic fibrosis, which will expand our understanding of the pathogenesis of the reduced bone mass seen in patients with this disease. Fifty subjects (25 male and 25 female) with CF between the ages of 18-30 years are being recruited through the Children's Hospital Boston (CHB) and the Massachusetts General Hospital (MGH) Cystic Fibrosis Centers to enroll in this cross-sectional study. Subjects undergo a laboratory evaluation and complete health, medication, and nutrition surveys at the MGH Clinical Research Center (CRC), followed by dual energy x-ray absorptiometry (DXA) and HRp-QCT scans at the MGH Bone Density Center. Five patients have currently completed this study. Once completed, data will be compared to healthy controls matched for age and gender.

2. SPECIFIC AIM AND HYPOTHESES

Specific Aim 1: To assess the change in bone micro-architecture, bone strength, and bone turnover markers over time in CF subjects treated with VX-770 (ivacaftor) compared to CF subjects not receiving this medication.
Primary Hypothesis: Total, cortical, and trabecular volumetric densities, as well as overall bone strength (estimated failure load) will be higher in subjects in subjects treated with VX-770 than in those who are not receiving this medication.
Secondary Hypothesis: Markers of bone formation will increase and markers of bone resorption will decrease in subjects treated with VX-770 medication compared to those who are not receiving this medication.

Specific Aim 2: To assess the change in bone micro-architecture, bone strength, and bone turnover markers over time in CF subjects not treated with VX-770 compared to healthy subjects.
Primary Hypothesis: Total, cortical, and trabecular volumetric densities, as well as overall bone strength (estimated failure load) will be lower in CF subjects and will decline over time compared to healthy subjects.
Secondary Hypothesis: Markers of bone formation will be lower and markers of bone resorption will be higher in CF subjects compared to healthy subjects.

3. SUBJECT SELECTION

3.1 Study population:

Three subject cohorts will be recruited as follows:

<u>Cohort 1:</u>
30 subjects ages 6 – 75 years will be recruited in accordance with the written guidelines of the Children's Hospital Boston and Massachusetts General Hospital Institutional Review Boards. Subjects will be required to meet the following criteria:

Inclusion criteria:

- Established diagnosis of CF with at least one abnormal G551D-CFTR allele
- Eligibility for and intent to start treatment with VX-770 or started treatment with VX-770 within previous 6 months

Exclusion criteria:

- Psychiatric or mental incapacity that would preclude subject from assenting to study participation
- Current pregnancy
- History of organ transplantation
- History of *Burkholderia dolosa* infection

Of note, if subjects initially are enrolled as a part of Cohort 1 but then do not start ivacaftor for any reason, then they can remain in the study and will be moved from Cohort 1 to Cohort 2.

Cohort 2:

30 subjects will be recruited in accordance with the written guidelines of the Children's Hospital Boston and Massachusetts General Hospital Institutional Review Boards. Subjects will be grouped by gender, age and race to match subjects in Cohort 1 within two years. Pubertal subjects will be matched by Tanner stage instead of age. Subjects will be required to meet the following criteria:

Inclusion criteria:

- Established diagnosis of CF

Exclusion criteria:

- Psychiatric or mental incapacity that would preclude subject from assenting to study participation
- Current pregnancy
- History of organ transplantation
- History of *Burkholderia dolosa* infection

Cohort 3:

30 subjects will be recruited in accordance with the written guidelines of the Children's Hospital Boston and Massachusetts General Hospital Institutional Review Boards. Subjects will be grouped by gender, age and race to match subjects in Cohort 2 within two years. Pubertal subjects will be matched by Tanner stage instead of age. Subjects will be required to meet the following criteria:

Inclusion criteria:

- Clinically stable, deemed able to complete the screening, baseline, and scheduled study visits.

Exclusion criteria:

- History of significant cardiac, renal, pulmonary, hepatic, or malignant disease, current alcohol or illicit drug abuse, or major psychiatric disorder
- Current diagnoses known to affect bone metabolism, including cystic fibrosis, osteoporosis, amenorrhea >3 months (in menstruating women who are not taking oral contraceptives or have an IUD), hyperthyroidism, diabetes, hyperparathyroidism,

Paget's disease, kidney stones, chronic inflammatory diseases, malabsorptive disorders, malnutrition, prolonged immobility, and skeletal dysplasias

- History of a non-digital fracture in the previous 6 months, history of one pathologic fracture, or greater than four total lifetime non-digital fractures
- Cumulative lifetime use of oral glucocorticoids for greater than 2 months
- Current or prior use of medications known to affect bone metabolism including hormone replacement therapy, anti-estrogens, bisphosphonates, calcitonin, fluoride, lithium, suppressive doses of levothyroxine, or anticonvulsants.
- Pregnancy
- BMI less than 18.5 or greater than 30 kg/m^2 in subjects 18 years and older, or BMI less than 5^{th} or greater than 95^{th} percentile in subjects under the age of 18 years.
- Any medical or psychiatric condition or situation that would compromise subject safety, informed consent/assent, treatment compliance, follow-up measurements, or data quality

3.2 Recruitment Strategy

Subjects with CF will be recruited from the Cystic Fibrosis Centers at Children's Hospital Boston and Massachusetts General Hospital. A brochure describing the study will be made available in each of the clinics. Potential subjects who have previously given their consent in writing to the CF Program granting permission to be contacted regarding research studies will be contacted by phone or email according to their previously indicated preference. In addition, potential subjects will be asked by their pulmonologist, pediatrician, or other health care provider during their regularly scheduled clinic visits if they are interested in participating in a research project. If they agree, then they will be approached by members of the research team either at the appointment or contacted by phone/email according to their preference. Potential subjects who are interested will be forwarded an informed consent form to review and will be invited to schedule a study visit.

Healthy subjects will be recruited from the community using flyers, postings, and advertisements using IRB-approved materials. Phone screening will be used to determine inclusion/exclusion criteria for potential subjects.

Subjects will receive $50 and reimbursement for transportation upon completion of each study visit.

A subset of subjects who have completed the study visit of current Protocol 2011P000893 ("Bone strength and micro-architecture in young adults with cystic fibrosis") will be eligible for participation in this proposed study. The study procedures for the study visit of Protocol 2011P000893 are identical to the baseline visit of the current proposed study. Therefore, if subjects in Protocol 2011P000893 are interested in also participating in the current proposed study, then their completed study visit will take the place of the baseline visit of this proposed protocol, and they will subsequently be re-consented for enrollment in the proposed study at Follow Up Visit 1 (12 months after the date of their protocol 2011P000893 study visit, or 12 months after starting VX-770 in

those in cohort 1). This will avoid any unnecessary radiation exposure and blood draws to subjects who desire to participate in both studies.

4. SUBJECT ENROLLMENT

4.1 Methods of enrollment

Potential subjects identified above will be scheduled for a research visit at the MGH CRC, at which time informed consent and assent will be obtained and subjects enrolled in the study.

4.2 Informed consent

Written informed consent will be obtained using a consent form that has been approved by the Partners Institutional Review Board before subjects undergo any study procedures other than asking simple questions to determine potential eligibility and interest in the study. Informed consent will be obtained by a licensed physician with the subject and parent and/or guardian as necessary for subjects under the age of 18 years. All subjects will be informed regarding the purpose of the research, the details of the study protocol, risks and benefits, alternatives to participation, costs, reimbursements, their right to privacy and confidentiality, their right to refuse to participate or withdraw from the study at any time, their rights in the event of a study-induced injury, and whom to contact for questions about the study. Subjects will be given a copy of their signed consent form and an additional copy will be kept in our research files. Children and adolescents under the age of 18 years will be asked to sign an assent form and their guardian will sign the consent form.

4.3 Treatment assignment and randomization

N/A

5. STUDY PROCEDURES

5.1 Protocol

Subjects will be seen at the MGH General Clinical Research Center (GCRC) for baseline visit and 12- and 24-month follow-up visits.

Baseline visit:

Baseline visit will be arranged within 6 months of initiation or anticipated initiation of VX-770 in Cohort 1. After obtaining informed consent, a survey will be administered to obtain information regarding medical history, hospitalizations in the previous 12 months, medication use over the previous 12 months, history of fractures, physical activity, and sunlight exposure. Most recent pulmonary function test results will be obtained from the medical record. Urine hCG will be performed for female subjects. Blood and urine will be collected for the tests listed below. Subjects will then be escorted to the MGH Bone Densitometry Center, where they will undergo DXA measurements of the PA and lateral spine, hip, radius, and total body and HR-pQCT measurements of the non-dominant distal radius and tibia.

CYSTIC FIBROSIS RELATED BONE DISEASE: THE ROLE OF CFTR
PI: Melissa Putman MD

1. Blood Studies
    a. Routine chemistry panel (including calcium, phosphate, magnesium, albumin, BUN, and creatinine)
    b. C-reactive protein (CRP)
    c. Parathyroid hormone (PTH)
    d. Insulin-like growth factor-1 (IGF-1)
    e. 25-hydroxyvitamin D [25(OH)D]
    f. Osteocalcin (OC)
    g. Bone-specific alkaline phosphatase (BSAP)
    h. C-telopeptides of type 1 collagen (CTX)
    i. Total testosterone (males only)
    j. Sex hormone binding globulin (SHBG), males only

    One extra tube of blood will be collected and stored in 0.5 mL aliquots in case any tests need to be repeated and for measurement of additional analytes of interest in the future.

    In subjects less than 18 years of age, no more than 3 cc/kg body weight of blood will be drawn per 8 week period.
2. Urine studies
    a. N-telopeptides of type 1 collagen (NTX)
    b. Urine HCG (females only)
3. Bone density and body composition by dual-energy x-ray absorptiometry (DXA)
    a. Whole lumbar vertebrae (DXA in the PA projection)
    b. Lumbar vertebral bodies (DXA in the lateral projection), subjects ≥18y only
    c. Proximal femur (femoral neck and total hip)
    d. Forearm (distal radius)
    e. Total body BMD
    f. Vertebral facture assessment (VFA) in adults ≥18y only
    g. Fat and lean mass
4. Bone micro-architecture by HR-pQCT (see below)
    a. Distal radius
        i. Standard site in adults
        ii. 7% site in children and adolescents <17y
    b. Tibia
        i. Standard site in adults
        ii. 8% site in children and adolescents <17y
5. Anthropometric measures (weight, height, BMI)
6. Physical Exam (pubertal children only) for Tanner staging
7. Medical history (from survey and/or review of patient medical chart)
    a. Fracture history (with X-ray confirmation if available)
    b. Hospitalizations in the previous 12 months
    c. Medication use in the previous 12 months
    d. Vitamin and other supplement use in the previous 12 months
    e. Lifetime oral glucocorticoid use
    f. Growth chart
    g. Sun exposure (using standardized scale) [40]
    h. Self identified race/ethnicity

i. Pubertal development
j. Menstrual history (in females)
k. Dietary history including vitamin D intake (24 hour food records)
l. Physical activity survey [41]
m. Most recent pulmonary function tests (FEV1) within the preceding 4-8 weeks in Cohorts 1 and 2
n. Results from previous genetic testing (if performed) regarding CFTR mutation type in Cohorts 1 and 2

Interventions
This is a non-interventional study. All pulmonary and endocrine clinical care will continue as per the discretion of subjects' primary clinical providers. VX-770 will be prescribed and managed by their primary pulmonologist.

Follow up Visits
Follow-up visits will take place 12 and 24 months after the baseline visit. This visit will include similar procedures and surveys as described in baseline visit, obtaining pertinent data over the previous 12 months. Because bone density changes happen very slowly and in order to accommodate subjects' schedules (including their medical care needs, school, relocation to other parts of the country, and other issues), study visits will be scheduled at 12 and 24 months with a window of +/- 3 months so as not to be overly prohibitive or coercive with scheduling. Data analysis will take into account length of time between follow up visits.

1. Blood Studies
    a. Routine chemistry panel (including calcium, phosphate, magnesium, albumin, BUN, and creatinine)
    b. CRP
    c. PTH
    d. IGF-1
    e. 25(OH)D
    f. OC
    g. BSAP
    h. CTX
    i. Total testosterone (males only)
    j. SHBG (males only)

    One extra tube of blood will be collected and stored in 0.5 mL aliquots in case any tests need to be repeated and for measurement of additional analytes of interest in the future.

    In subjects less than 18 years of age, no more than 3 cc/kg body weight of blood will be drawn per 8 week period.
2. Urine studies
    a. NTX
    b. Urine HCG (females only)
3. Bone density and body composition by dual-energy x-ray absorptiometry (DXA)
    a. Whole lumbar vertebrae (DXA in the PA projection)
    b. Lumbar vertebral bodies (DXA in the lateral projection), subjects ≥18y only

   c. Proximal femur (femoral neck and total hip)
   d. Forearm (distal radius)
   e. Total body BMD
   f. Vertebral facture assessment (VFA) in adults >17 only (at 24 month follow up visit only)
   g. Fat and lean mass
4. Bone micro-architecture by HR-pQCT (see below)
   a. Distal radius, using same site as baseline visit
   b. Tibia, using same site as baseline visit
5. Anthropometric measures (weight, height, BMI)
6. Physical Exam (pubertal children only) for Tanner staging
7. Medical history (from survey and/or review of patient medical chart) over past 12 months
   a. Fracture history in previous 12 months (with X-ray confirmation if available)
   b. Hospitalizations in the previous 12 months
   c. Medication use, including glucocorticoids, in the previous 12 months
   d. Vitamin and other supplement use in the previous 12 months
   e. Growth chart
   f. Sun exposure (using standardized scale) [40]
   g. Pubertal development
   h. Menstrual history (in females)
   i. Dietary history including vitamin D intake (24 hour food records)
   j. Physical activity survey [41]
   k. Most recent pulmonary function tests (FEV1) within the preceding 4-8 weeks in Cohorts 1 and 2
   l. Self report of compliance with VX-770 (Cohort 1 only)

Subjects in cohorts 1 and 2 and/or their insurance companies will not be billed for any procedure that is not standard of care for patient with CF. Subjects in cohort 3 and/or their insurance companies will not be billed for any study procedures.

Study Procedure Schematic

	StudyVisit 1	**StudyVisit 2**	**Study Visit 3**
Month	**0**	**12**	**24**
Consent	**X**	**X (in participants of protocol 2011P000893 only)**	
Medical history, Tanner staging (pubertal children only)	**X**	**X**	**X**
Fracture history, most recent FEV1, medication use, physical activity survey	**X**	**X**	**X**

Height, weight	**X**	**X**	**X**
Ca and vitamin D intake questionnaire	**X**	**X**	**X**
Labs: 25OHD, Ca, alb, creatinine, Mg, PTH, IGF-1, CRP, testosterone+SHBG (males), bone turnover markers, βHCG (females)	**X**	**X**	**X**
DXA scan (total body, hip, spine, wrist)	**X**	**X**	**X**
DXA VFA (subjects ≥ 18 years only)	**X**		**X**
HR-pQCT radius and tibia	**X**	**X**	**X**

5.1.1 Additional information on high resolution peripheral computed tomography (Xtreme CT)

XtremeCT (Scanco Medical AG, Basserdorf, Switzerland) is a new technology capable of assessing volumetric bone density as well as trabecular and cortical micro-architecture at the distal radius and distal tibia, with a nominal isotropic voxel size of 82 µm. The XtremeCT system (Figure 1) employs a two-dimensional detector array in combination with a small-angle cone beam X-ray source (0.07 mm spot focal size), enabling the simultaneous acquisition of a stack of parallel CT slices with a nominal resolution (voxel size) of 82 µm (100 µm at 10% MTF). The X-ray tube employs an effective energy of 60 kVp, with a current of 95 mAs. The field of view is 130 mm and maximum scan length is 150 mm. The short-term reproducibility of measurements with this device is excellent, with precision errors of 0.7 to 1.5% for total, trabecular and cortical bone densities and 2.5 to 4.4% for trabecular architecture measures.

Scan acquisition: During scan acquisition, the arm or leg of the patient is immobilized in an anatomically formed carbon fibre shell. An antero-posterior scout view is used to define the measurement region. At each skeletal site, 110 CT slices are acquired, thus delivering a 3D representation of approximately 9 mm in the axial direction. In standard analyses, a reference line is manually placed at the endplate of the radius and tibia, as shown in Figure 2 (middle and right). The first CT slice is acquired 9.5 mm and 22.5 mm proximal to the reference line for the distal radius and distal tibia, respectively (Figure 2). In subjects under 17 years of age, the reference line will be set at the 8% site at the radius and 7% site at the tibia in order to avoid radiation to the growth plate, based on previously published data using Xtreme CT in children and adolescents.[42-43]

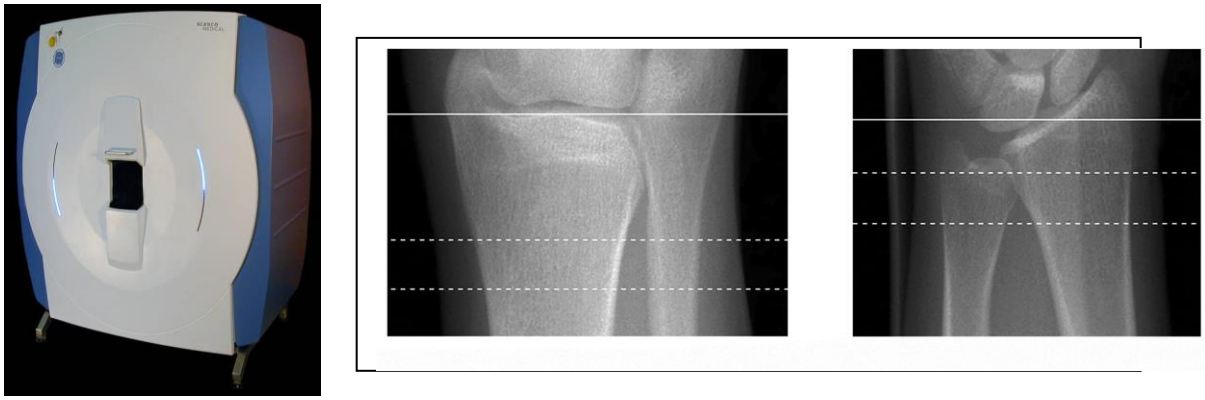

Figure 2. (left) Photo of XtremeCT clinical imaging device, for assessment of trabecular and cortical bone microarchitecture at the distal radius and distal tibia. Image courtesy of Scanco Medical, AG. The middle and right images are standard antero-posterior scout views of the distal tibia (middle) and distal radius (right), showing the placement of a reference line (solid line) and the volume of interest where CT slices are acquired (between dashed lines).

The following data will be obtained at both sites: total bone area, cortical area, trabecular area, total volumetric bone density, cortical volumetric bone density, cortical thickness, trabecular volumetric bone density, trabecular bone volume fraction (BV/TV), trabecular number, trabecular separation, trabecular thickness, cortical porosity, and estimated bone strength (calculated microfinite element).

6. BIOSTATISTICAL ANALYSIS

6.1 Study Outcomes/Endpoints

The primary outcome of specific aim 1 will be the difference in the mean change in cortical volumetric density of the radius and tibia as measured by HR-pQCT over 24 months between cohorts 1 and 2. The primary outcome of specific aim 2 will be the difference in the mean change in cortical volumetric density of the radius and tibia as measured by HR-pQCT over 24 months between cohorts 2 and 3. Secondary endpoints will include change in total and trabecular volumetric density, as well as other HR-pQCT parameters (trabecular number, trabecular spacing, cortical porosity, and microfinite element of the radius and tibia), DXA values (BMD of spine, hip, radius, and total body), and bone turnover markers.

6.2 Statistical Methods

Statistical analysis will include descriptive statistics, with determination of means and SD of change in each primary and secondary outcome variable from baseline to 12 and 24 months for all three cohorts. For specific aim 1, mean change in primary outcome in cohorts 1 and 2 will be compared with independent-sample t-tests (or Wilcoxon rank sum if non-normality is detected). For specific aim 2, mean change in primary outcome in cohorts 1 and 2 will be compared with independent-sample t-tests (or Wilcoxon rank sum if non-normality is detected). Longitudinal linear mixed models will be applied to evaluate longitudinal changes in BMD, bone microarchitectural parameters, and bone turnover markers over the 2-year period in each group. Length of time between follow up visits will be accounted for in statistical analyses. Statistical analysis will be performed in consultation with a study statistician.

6.3 Sample Size Considerations:

Sample-size calculations were performed after consultation with our biostatistician using data from 103 healthy adult controls previously scanned using HR-pQCT at the MGH Bone Density Center as well as previously published reproducibility data.[31] A sample size of Cohort 1 n=30 and Cohort 2 n=30 would provide 80% power (Type II error = 0.2) to detect an effect on the primary outcome variable (mean change in cortical volumetric density of the radius and tibia over 24 months) of 2.3-3.1% using a pre-post correlation of 0.7. Based on numbers of CF patients both with and without G551D mutations treated at the MGH and CHB CF Centers, we will have adequate access to subjects to meet this sample size requirement.

BETWEEN GROUP CHANGE

	n1	n2	Radius Mean	Radius SD	SD change over time Within group	Detectable dif in mean change over 1yr (Radius) *	% dif	Tibia Mean	Tibia SD	SD change over time Within group	Detectable dif in mean change over 1yr (Tibia)*	% dif
1° OUTCOME												
Cortical vBMD	10	30	856.7	47.19	36.34	33.04	3.86	892.6	36.07	27.77	25.26	2.83
	20	30	856.7	47.19	36.34	29.42	3.43	892.6	36.07	27.77	22.49	2.52
	30	30	856.7	47.19	36.34	26.73	3.12	892.6	36.07	27.77	20.43	2.29
2° OUTCOMES												
Total vBMD	10	30	343.3	57.53	44.30	40.28	11.73	342.5	48.96	37.70	34.28	10.01
	20	30	343.3	57.53	44.30	35.87	10.45	342.5	48.96	37.70	30.52	8.91
	30	30	343.3	57.53	44.30	32.59	9.49	342.5	48.96	37.70	27.73	8.10
Trab vBMD	10	30	193.9	39.77	30.62	27.85	14.36	208.9	38.86	29.92	27.21	13.03
	20	30	193.9	39.77	30.62	24.80	12.79	208.9	38.86	29.92	24.23	11.60
	30	30	193.9	39.77	30.62	22.53	11.62	208.9	38.86	29.92	22.01	10.54
Cort Thickness	10	30	0.83	0.19	0.14	0.13	15.53	1.3	0.26	0.20	0.18	14.08
	20	30	0.83	0.19	0.14	0.12	13.84	1.3	0.26	0.20	0.16	12.54
	30	30	0.83	0.19	0.14	0.10	12.52	1.3	0.26	0.20	0.15	11.38
Trab Thickness	10	30	0.078	0.013	0.0100	0.009	11.59	0.084	0.013	0.0097	0.008	9.51
	20	30	0.078	0.013	0.0100	0.008	10.30	0.084	0.013	0.0097	0.007	8.32
	30	30	0.078	0.013	0.0100	0.007	9.01	0.084	0.013	0.0097	0.007	8.32

* Using Type 1 error 0.05 and 80% power, assuming pre-post correlation 0.7

7. Potential pitfalls

Although a randomized control trial would be the ideal study design to evaluate the effects of VX-770 on bone, we believe it would be unethical to withhold this medication from subjects who qualify for it given its previously established efficacy and the nature of this ultimately fatal disease. Therefore, we propose a prospective multiple cohort study, using subjects with CF who are not eligible for this medication (i.e. have CF mutations other than G551D) and healthy subjects without CF. Although this will necessitate inherent genetic differences between CF cohorts, there are no animal studies that confirm phenotypic bone differences between G551D in particular and other genotypes. One study by King et al suggested that adult CF patients with DF508 mutations have lower bone density than those without this mutation, though this was limited by its cross sectional design and did not specifically compare subjects with G551D mutations. Further studies on genotype-phenotype bone differences are required.

Because of this study design, confounders may exist between cohorts. In addition, if lung and other organ function improve with VX-770, this may independently affect bone density apart from the direct effects of the drug on bone. Therefore, we will plan to collect data regarding physical activity, sun exposure, medication use (including glucocorticoids), gonadal function (menstrual history in women, morning testosterone levels in men), inflammatory markers, PTH/vitamin D, BMI, and fat/lean body mass by DXA. We will adjust for potential confounders that are significantly difference between cohorts in our multivariate analysis.

Data have not confirmed that VX-770 will affect CFTR function in bone cells to the extent that it affects epithelial cells, or that it is absorbed in bone to an appreciable effect. To test this hypothesis, we have proposed a separate study to add bone turnover makers to saved sera from the previous VX-770 phase 3 clinical trial[25] to determine if changes in bone resorption and formation occurred over the course of the year-long study. If significant changes in bone turnover are found, this will add further support to the present proposal.

7. REFERENCES

1. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, Guise TA, Hardin DS, Haworth CS, Holick MF, Joseph PM, O'Brien K, Tullis E, Watts NB, White TB. Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab 2005; 90:1888-96.

2. Conway SP, Morton AM, Oldroyd B, Truscott JG, White H, Smith AH, Haigh I. Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors. Thorax 2000; 55: 798-804.

3. Bianchi ML, Romano G, Saraifoger S, Costantini D, Limonta C, Colombo C. BMD and body composition in children and young patients affected by cystic fibrosis. J Bone Min Res 2006; 21: 388-96.

4. Grey V, Atkinson S, Drury D, Casey L, Ferland G, Gundberg C, Lands LC. Prevalence of low bone mass and deficiencies of vitamin D and K in pediatric patients with cystic fibrosis from 3 Canadian centers. Pediatrics 2008; 122:1014-20.

5. Haworth CS, Selby PL, Horrocks AW, Mawer EB, Adams JE, Webb, AK. A prospective study of change in bone mineral density over one year in adults with cystic fibrosis. Thorax 2002; 57:719-23.

6. Paccou J, Zeboulon N, Combscure C, Gossec L, Cortet B. The prevalence of osteoporosis, osteopenia, and fractures among adults with cystic fibrosis: A systematic literature review with meta-analysis. Calcif Tissue Int 2010; 86:1-7.

7. Gordon CM, Binello E, LeBoff MS, Wohl ME, Rosen CJ, Colin AA. Relationship between insulin-like growth factor 1, dehydroepiandrosterone sulfate and pro-resorptive cytokines and bone density in cystic fibrosis. Osteoporos Int 2006; 17:783-90.

8. Aris RM, Ontjes DA, Buell HE, Blackwood AD, Lark RK, Caminiti M, Brown SA, Renner JB, Chalermskulrat W, Lester GE. Abnormal bone turnover in cystic fibrosis adults. Osteoporos Int 2002; 13:151-7.

9. Aris RM, Renner JB, Winders AD, Buell HE, Riggs DB, Lester GE, Ontjes DA. Increased rate of fractures and severe kyphosis: sequelae of living to adulthood with cystic fibrosis. Ann Intern Med 1998; 128:186-93.

10. Stephenson A, Jamal S, Dowdell T, Pearce D, Corey M, Tullis E. Prevalence of vertebral fractures in adults with cystic fibrosis and their relationship to bone mineral density. Chest 2006; 130:539-44.

11. Henderson RC, Specter BB. Kyphosis and fractures in children and young adults with cystic fibrosis. J Pediatr 1994; 125:208-12.

12. Rossini M, Del Marco A, Dal Santo F, Gatti D, Braggion C, James G, Adami S. Prevalence and correlates of vertebral fractures in adults with cystic fibrosis. Bone 2004; 35:771-6.

13. Erkkila J, Warwick W, Bradford D. Spine deformities and cystic fibrosis. Clin Orthop 1978; 131:146-9.

14. Logvinoff M, Fon G, Taussig LM, Pitt MJ. Kyphosis and pulmonary function in cystic fibrosis. Clin Pediatr 1984; 23:389-92.

15. Gordon CM, Anderson EJ, Herlyn K, Hubbard JL, Pizzo A, Gelbard R, Lapey A, Merkel PA. Nutrient status in adults with cystic fibrosis. J Am Diet Assoc 2007; 107:2114-7.

16. King SJ, Topliss DJ, Kotsimbos T, Nyulasi IB, Bailey M, Ebeling PR, Wilson JW. Reduced bone density in cystic fibrosis: Delta F508 mutation is an independent risk factor. Eur Respir J 2005; 25:54-61.

17. Bhudhikanok GS, Wang MC, Marcus R, Harkins A, Moss RB, Bachrach LK. Bone acquisition and loss in children and adults with cystic fibrosis: a longitudinal study. J Pediatr 1998; 133:18-27.

18. Bronckers A, Kalogeraki L, Jorna HJN et al. The cystic fibrosis transmembrance conductance regulator (CFTR) is expressed in maturation stage ameloblasts, odontoblasts, and bone cells. Bone 2010; 46: 1188-96.

19. Dif F, Marty C, Baudoin C et al. Severe osteopenia in CFTR-null mice. Bone 2004; 35:595-603.

20. Haston CK, Li w, Li A et al. Persistent osteopenia in adult cystic fibrosis transmembrane conductance regulator-deficient mice. Am J Respir Crit Care Med 2008; 177:309-15.

21. Shead EF, Haworth CS, Condliffe AM et al. Cystic fibrosis transmembrance regulator (CFTR) is expressed in human bone. Thorax 2007; 62:650-1.

22. Le Heron L, Guillaume C, Velard F et al. Cystic fibrosis transmembrance conductance regulator (CFTR) regulates the production of osteoprotegerin (OPG) and prostaglandin (PG) E2 in human bone. J Cyst Fibr 2010; 9:69-72.

23. Elkin SL, Vedi S, Bord S, et al. Histomorphometric analysis of bone biopsies from the iliac crest of adults with cystic fibrosis. Am J Resp Crit Care Med 2002; 166:1470-4.

24. Accurso FJ, Rowe SM, Clancy JP et al. Effect of VX-770 in Persons with Cystic Fibrosis and the G551D-*CFTR* Mutation. N Engl J Med 2010; 363:1991-2003.

25. Ramsey BW, Davies J, McElvaney NG et al. A CFTR Potentiator in Patients with Cystic Fibrosis and the *G551D* Mutation. New Engl J Med 2011; 365:1663-72.

26. Ulrich D, van Rietbergen B, Laib A, Ruegsegger P. The ability of three dimensional structural indices to reflect mechanical aspects of trabecular bone. Bone 1999; 25:55-60.

27. Jarvinen T, Kannus P, Sievanen H. Have the DXA-based exercise studies seriously underestimated the effects of mechanical loading on bone? J Bone Min Res 1999; 14:1634-5.

28. Louis O, Clerinx P, Gies I, De Wachter E, De Schepper J. Well-nourished cystic fibrosis patients have normal bone mineral density, but reduced cortical thickness at the forearm. Osteoporos Int 2009; 20:309-14.

29. MacNeil JA, Boyd SK. Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality. Med Eng Phys 2007; 29:1096–105.

30. Boyd S. Site-Specific Variation of Bone Micro-Architecture in the Distal Radius and Tibia. J Clin Den 2008; 11:424-30.

31. Boutroy S, Bouxsein M, Munoz F, Delmas PD. In Vivo Assessment of Trabecular Bone Microarchitecture by High-Resolution Peripheral Quantitative Computed Tomography. JCEM 2005; 90:6508-15.

32. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: An in vivo HR-pQCT study. J Bone Min Res 2010; 25:882-90.

33. Bacchetta J, Boutroy S, Vilayphiou N, Juillard L, Guebre-Egziabher F, Rognant N,Sornay-Rendu E, Szulc P, Laville M, Delmas PD, Fouque D, Chapurlat R. Early impairment of trabecular microarchitecture assessed with HR-pQCT in patients with stage II-IV chronic kidney disease. J Bone Min Res 2009; 25:849-57.

34. Sornay-Rendu E, Boutroy S, Munoz F, and Delmas PD. Alterations of Cortical and Trabecular Architecture Are Associated With Fractures in Postmenopausal Women, Partially Independent of Decreased BMD Measured by DXA: The OFELY Study. J Bone Min Res 2007; 22:425-33.

35. Vico L, Zouch M, Amirouche A, Frère D, Laroche N, Koller B, Laib A, Thomas T, Alexandre C. High-Resolution pQCT Analysis at the Distal Radius and Tibia Discriminates Patients With Recent Wrist and Femoral Neck Fractures. J Bone Min Res 2008; 23:1741-50.

36. Burghardt AJ, Issever AS, Schwartz AV, Davis KA, Masharani U, Majumdar M, Link TM. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. J Clin Endocrin Metab 2011; 95:5045-55.

37. Burghardt AJ, Kazakia GJ, Sode M, de Papp AE, Link TM, Majumdar S. A longitudinal HR-pQCT study of alendronate treatment in postmenopausal women with low bone density: Relations among density, cortical and trabecular microarchitecture, biomechanics, and bone turnover. J Bone Miner Res 2010: 25:2558-71

38. Macdonald HM, Nishiyama KK, Hanley DA, Boyd SK. Changes in trabecular and cortical bone microarchitecture at peripheral sites associated with 18 months of teriparatide therapy in postmenopausal women with osteoporosis. Osteoporos Int 2011; 22:357-62.

39. Seeman E, Delmas PD, Hanley DA, Sellmeyer D, Cheung AM, Shane E, Kearns A, Thomas T, Boyd SK, Boutroy S, Bogado C, Majumdar S, Fan M, Libanati C, Zanchetta J. Microarchitectural deterioration of cortical and trabecular bone: differing effects of denosumab and alendronate. J Bone Miner Res 2010; 25:1886-94.

40. Kriska AM and Bennett PH. An epidemiological perspective of the relationship between physical activity and NIDDM: from activity assessment to intervention. Diabetes Metab Rev 1992; 8:355-372.

41. Salamone LM, Dallal GE, Zantos D, Makrauer F, Dawson-Hughes B. Contributions of vitamin D intake and seasonal sunlight exposure to plasma 25-hydroxyvitamin D concentration in elderly women. Am J Clin Nutr 1994; 59:80-86.

42. Liu D, Burrows M, Egeli D, McKay H. Site Specificity of Bone Architecture Between the Distal Radius and Distal Tibia in Children and Adolescents: An HR-pQCT Study. Calcif Tissue Int 2010; 87:314-323.

43. Burrows M, Liu D, Perdios A, Moore S, Mulpuri K, McKay H. Assessing bone microstructure at the distal radius in children and adolescents using HR-pQCT: a methodological pilot study. J Clin Densitom 2010; 13: 451-5.