TITLE OF PROJECT

OSTEOPOROSIS IN CYSTIC FIBROSIS: STUDY OF BONE MASS AND BONE METABOLISM, AND PROSPECTIVE RANDOMIZED THERAPEUTIC TRIAL

SYNOPSIS

Early development of osteoporosis has been documented in many chronic diseases in children, adolescents and young adults, where nutritional deficiencies, delayed puberty or the chronic use of corticosteroids and other drugs may have a pathogenetic role in the abnormally low mineralization of bone. Research data about the clinical evaluation and treatment of osteoporosis are however still lacking for this age group.

Cystic fibrosis (CF) is an autosomal recessive genetic disease which affects about 60,000 individuals worldwide, including about 3,800 in Italy, and which is often associated with low bone mass. Together with a much longer survival of CF patients, the very success of current aggressive therapies leads to a greater number of cases of osteoporosis and bone fractures, a serious problem which not only affects quality of life, but also hinders further therapeutic measures.

The aim of the proposed research is the study of bone mass changes in a large group of children, adolescents and young adults affected by cystic fibrosis, and the feasibility and efficacy of osteoporosis treatment in such patients. Following baseline evaluation, bone mass changes will first be studied over 1 year with a simple therapy of adequate calcium intake and 25-OH vitamin D supplements. Cases showing an insufficient response will then be submitted - in addition to calcium and 25-OH vitamin D - to a prospective, double-blind, randomized, placebo-controlled 1-year trial of alendronate treatment. This will be the first large study of osteoporosis in young patients with cystic fibrosis.

It will be carried out by the Coordinator's Institution in collaboration with most Regional Reference Centres for cystic fibrosis in Italy, involving an estimated 250 patients.

THERAPEUTIC PERSPECTIVE

Evaluation of efficacy/safety of correct calcium intake, 25OH D3, alendronate therapy in osteoporosis (OP) in patients with cystic fibrosis (CF). If successful, a standardized diagnostic and therapeutic protocol for OP management in CF could be developed.

RELEVANCE FOR TELETHON

Assessment of a new therapeutic strategy for osteoporotic complications in cystic fibrosis (CF), with likely advantages on eligibility to lung transplant and improvement of the patients' quality of life while they are waiting for a better CF therapy.

INTRODUCTION AND RATIONALE

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CFTR gene (1). Approximately 60,000 individuals are affected worldwide, about 3,800 in Italy (2). There is no cure and survival depends on continuous supportive treatment: CF is generally severe and leads to death for respiratory insufficiency. Beyond pulmonary, there are pancreatic, hepatic, intestinal, and other complications. New aggressive therapeutic strategies led to a significantly increased survival, with many patients reaching mature age. However, this survival has caused the emergence of long-term sequelae such as renal damage, diabetes and osteoporosis.

Osteoporosis (“A systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk” (3)) is a common disease in post-menopausal women. Alendronate (a bisphosphonate) (4-6) is one of the most widely used drugs in osteoporosis. Early development of osteopenia or osteoporosis has been documented in many chronic diseases in children (e.g. chronic nephropathies, juvenile rheumatoid arthritis, diabetes mellitus, CF, etc.), where nutritional deficiencies, reduced mobility, delayed puberty or the chronic use of corticosteroids and other drugs may have a role in an abnormally low bone mineralization. Research data about the clinical evaluation and treatment of osteoporosis are still lacking for this age group, and there is only a small number of case reports and uncontrolled studies. The use of bisphosphonates has been prevalently studied in children with osteogenesis imperfecta, in uncontrolled experiences (7-12). Only a few studies on osteoporosis have been carried out in young patients with CF.
Many epidemiological studies in adults have shown that the value of bone mineral density (BMD) predicts the risk of bone fractures, and prospective studies have proved that such risk is inversely proportional to BMD (13-18). The peak value of bone mass, normally attained in young adult age, is considered the correct reference value in predicting the fracture risk for older people.

In recent years, following the availability of DXA, the process of mineral acquisition in the first decades of life has been increasingly studied, as there is hope that interventions aimed at maximizing the peak value of bone mass may prevent the late development of osteoporosis. Special attention should be paid to the young affected by the chronic diseases which carry an increased risk of bone mass impairment. Presently, there are no epidemiological studies relating BMD to the actual risk of fractures in young patients.

Rationale

The longer survival of CF patients raises the problem of preventing and treating long-term complications, including skeletal ones. Osteoporosis is frequent in CF (19-37), even in childhood: intestinal malabsorption, malnutrition, pubertal delay, hypogonadism, steroid treatment, hypo-oxygenation and poor physical activity have been implied as causal factors. Some risk factors are known, but other need to be ascertained. Bone fractures lead to reduced mobility and an increased risk of pulmonary infections. Severe bone disease may hinder some therapeutic approaches (e.g. transplants), reducing further survival. Rib and vertebral fractures due to osteoporosis are a serious problem in CF patients undergoing lung transplantation, and may limit the possibility of respiratory physical treatments, upon which CF patients largely depend. There are no known studies on the prevention and treatment of osteoporosis in CF.

The results of a collaborative study with the Lombardy Regional Reference Centre indicate that osteopenia and osteoporosis are a growing problem in CF patients. Not only many of them are at risk for bone fractures, but an increasing number of them actually suffer fractures.

Objectives

The primary objective of the project is to develop a therapeutic protocol for osteoporosis with a prospective study in a large group of children and young adults affected by cystic fibrosis (CF).

This will be accomplished in two steps:
-- a 1-year open trial on the effect of increasing calcium intake to RDA and administering 25-OH vitamin D as a first-line treatment;
-- a 1-year double-blind, randomized, placebo-controlled, prospective therapeutic trial of alendronate in patients showing persistent or aggravating osteoporosis, notwithstanding treatment with adequate calcium intake and 25-OH vitamin D.

The results will be evaluated in terms of clinical efficacy (bone mass increase), safety, frequency and importance of adverse effects.

The study will also address the following key aspects as secondary objectives:
-- evaluation of bone mass in a large population of CF patients from all over Italy (mass screening to enroll 250 subjects in the study);
-- bone mass determinants in CF;
-- biochemical markers of bone metabolism in CF.

The relevance of this study is mainly in the development of therapeutic strategies for the bone complications of CF, which, until now, has not been sufficiently addressed by clinical research, probably because CF is a rare genetic disease and its study can only be pursued in a not-for-profit research setting.

If the assessment of bone status and metabolism proves that the proposed therapeutic measures are successful in slowing the progression of osteoporosis and in reducing the fracture risk, then a standardized diagnostic and therapeutic protocol for the management of osteoporosis in CF could be developed. Preventing or treating osteoporosis, recognizing critical stages of bone mineralization during growth and development and identifying patients at significant risk will substantially ameliorate the CF patients’ quality of life, optimize therapeutic interventions, and possibly offer better chances of transplantation.

This is the first controlled trial of oral alendronate therapy of osteoporosis in a large young population with CF, and the feasibility and efficacy of alendronate treatment in such a clinical condition and age range will be investigated. In case of positive results, a similar therapeutic approach could be applied (at least partially) also to other systemic diseases known to induce metabolic bone alterations.

Studies on adult patients demonstrated that those with end-stage lung disease are at high risk for osteoporosis, and bone fragility fractures are often seen after lung transplant, particularly in the first year (38-41). The importance of a correct early recognition of “pathological” bone mass levels and the urgent need for an effective therapeutic approach is further stressed by the fact that in the Italian Lung Transplant Centres (Roma, Pavia, Padova) severe osteoporosis with bone fragility fractures is an exclusion criterion in the selection of patients. This
threshold for osteopenia is set at expressed in T-scores. According to WHO (42) and to the European Foundation for Osteoporosis (43), the threshold for osteopenia is set at -1 T-score and that for osteoporosis at -2.5 T-scores. Up to -1 T-score, the risk means that in the presence of osteoporosis, many children and young patients may be denied access to pulmonary transplant, their only hope to survive.

This single point should by itself justify the importance and urgency of launching a national study, aimed at evaluating the efficacy of the available therapeutic drugs for the prevention and treatment of osteoporosis in CF. Moreover, this point is strictly linked to another consideration that was clearly present during the preparing phase of this project. There is a growing need for clinical studies not only scientifically correct but also able to respond to the patients’ problems in a relatively short time. This project is not centered on the long-term strategies to treat CF, but on how to treat a severe complication of this disease as soon as possible to prevent further damages. It is very important for the patients and their quality of life to try to avoid a complication such as osteoporosis while they are waiting for a better therapy for CF.

As cystic fibrosis is a rare condition, in order to gather a significantly large population for the study, we have ensured the joint effort of several centres to obtain a sample of 250 patients.

On the basis of our previous investigations, we are confident to reach both the primary and secondary objectives of this research project. We would like to briefly present some preliminary results - some of them recently submitted for publication.

The Bone Metabolic Unit of the Istituto Auxologico Italiano IRCCS has been involved by the Lombardy Centre for Cystic Fibrosis in the development of diagnostic protocols and preliminary therapeutic strategies for osteoporosis in the last three years. Bone mineral density (BMD, mg/cm2) was systematically measured in patients affected by CF who supposedly presented risk factors for osteoporosis. BMD was measured at lumbar spine (L2-L4) and whole skeleton with dual X-ray absorptiometry (DXA) using a Hologic QDR 2000 device. BMD data are currently available for 162 patients (78 females, 84 males; mean age 17±8 years) representing about 1/3 of the total population followed at this centre. Our data showed a reduced BMD in many patients: in fact, only 34% of the patients had a normal BMD (Z score between 0 and -1), 27% were osteopenic (Z score between -1 and -2) and 39% were osteoporotic (Z score < -2). Step-wise multivariate analysis showed that BMD was positively related to an untreated control group. The BMD data, also expressed as Z-score, showed a clear improvement in the responding (baseline Z-score -3.3±0.9; after 12 months of therapy Z-score -2.4±0.4) while in the untreated group (43 patients) 74% had a decrease in BMD (baseline Z-score -2.7±1; after 12 months Z-score -3.5±8). In the treated group, 43% of the treated patients were non-responders: of these, 27% had a stable BMD (a negative result given their age), and 16% even had a decrease.

These preliminary data have been presented at: European Congress on Osteoporosis (Berlin, September 11-15, 1998); 2nd Annual North American Cystic Fibrosis Conference (Montreal, October 15-18, 1998); 21st ASBMR Meeting (St.Louis September 30-October 4, 1999).

STUDY DESIGN

This is a collaborative study involving 12 Regional Reference Centres (RRC) for cystic fibrosis. All the Italian centres have been contacted and no selection has been made. The participation of each centre depends exclusively on its willingness to share the research effort and on the local availability of the diagnostic equipment (DXA devices). All participating Centres have been required to perform a systematic search for CF patients with osteoporosis.

250 patients (age range 2-30 years) will be enrolled: as better explained in the following “Schedule” section (under the heading “Statistical analysis – sample size”), this number should ensure that enough patients will enter the second part of the study.

BMD is normally distributed, so it is possible to express it in terms of standard deviations (SD) from the mean of the reference population. A subject’s deviation from the mean of a normal population of the same sex is expressed in T-scores. According to WHO (42) and to the European Foundation for Osteoporosis (43), the threshold for osteopenia is set at -1 T-score and that for osteoporosis at -2.5 T-scores. Up to -1 T-score, the risk
of fractures is not significantly increased. Patients with a BMD value below -1.0 and -2.5 T-scores (osteopenia) have a moderate risk of fracture and should be considered for preventive measures. Patients with BMD below -2.5 T scores (osteoporosis) have a high risk of fracture and should be carefully followed and treated. In defining the threshold for osteopenia and osteoporosis in patients who have not yet reached the peak value of bone mass, the correct reference population is that of healthy children of the same sex and age. Accordingly, in our study, we considered the Z-scores (i.e. the subjects’ deviations from the mean of a healthy population normalized for sex and age) instead of the T-scores.

We conservatively defined osteoporosis as a lesser deviation from the mean in younger patients, considering the special significance of bone loss in growing children and adolescents and their smaller chance of ever attaining a satisfactory peak of BMD. The threshold for osteopenia was thus set at -1 Z-score, and that for osteoporosis at -2 Z-scores for patients aged less than 18 years.

Thus, all patients with a BMD Z-score \( \leq -2 \) (age up to 18 years) or \( \leq -2.5 \) (age over 18 years) will be considered eligible for the study, as well as all the patients with a history of fractures due to bone fragility. Routine laboratory tests will be made at each Centre’s facilities. Bone markers and calciotropic hormones will be centrally measured at the laboratory of the Istituto Auxologico Italiano IRCCS in Milan. DXA scans will also be centrally evaluated, within a quality control protocol, by the Coordinator at the Istituto Auxologico Italiano IRCCS in Milan. The study will last three years in total. The patients will be enrolled within the first 6 months. Two full years of observation and treatment are planned for each patient, so that the study will be closed at about the sixth month of the third year for the last patients enrolled. The results will be analyzed within the last 6 months.

ROLE OF THE COORDINATOR’S ORGANIZATION

The Coordinator has designed the project in cooperation with the staff of the Lombardy Reference Centre for CF of the Pediatric Department of the University of Milano. The Coordinator and her team at the Istituto Auxologico Italiano have developed all the materials for the study: informed consent letter, paper forms for clinical and follow-up data, including adverse event data; suggestions for improving dietary calcium intake; special instructions for the patients. A manual for DXA has been specially prepared to follow a standardized procedure for scanning and analyzing the results in each Centre. Also, detailed instructions for the collection of serum and urine samples to be centrally analyzed have been prepared. Throughout the study, the Coordinator will be responsible for the project management, the collection of all data and their entry in a computer data base. As specified above, the laboratory of the Istituto Auxologico Italiano will be responsible for all the centralized blood and urine tests. The Coordinator and her team will be responsible for the quality control of all BMD data. In our study, the final goal requires the most accurate evaluation of BMD changes. Therefore, all the DXA scans performed at the participating Centres will be centralized at the Istituto Auxologico Italiano, where a highly trained medical researcher - in a blind condition with respect to therapy - will verify their compliance with the predefined standards. Bone scans not attaining the requested quality will be rejected. In the last phase of the study, the Coordinator will analyze all the results with the help of professional statisticians.

ROLE OF PARTICIPATING CENTRES

All the participating centres will be responsible for the selection, enrollment and follow-up of their patients. Each centre will be responsible for the proper collection of clinical data, respect of the study schedule, monitoring the patients’ compliance, and rigorously reporting any adverse events; and will take care of local laboratory tests on blood and urine and BMD measurements. Each centre will be responsible for the handling of blood and urine samples for centralized analyses.

INCLUSION CRITERIA

All the patients followed at the Regional Referral Centres for CF, with:
-- age between 2 and 30 years;
-- a diagnosis of cystic fibrosis, based on abnormal results in at least two chloride sweat tests (according to the Gibson and Cook methods) and/or the presence of two CFTR mutations;
-- a diagnosis of osteoporosis (defined as BMD Z-score \( \leq -2 \) for patients up to 18 years, or \( \leq -2.5 \) for those over 18 years) or a history of fragility fractures.

EXCLUSION CRITERIA

Any patient with:
-- two or more episodes of hypercalcemia (serum calcium >10.5 mg/dl) and/or hypercalciuria (urinary Ca/Cr >0.3) or any other clinical conditions forbidding the use of active metabolites of vitamin D
-- esophageal varices, esophagitis, gastritis, duodenitis, gastroesophageal reflux
CONSENT

Informed consent will be obtained from the parents or tutors of all the eligible patients under 18 years of age; all eligible patients over 18 years will be asked to sign their informed consent.

ENROLMENT

During this phase, the eligible patients will be submitted to a first measurement of bone mineral density (BMD) at lumbar spine in order to calculate the initial Z-score and to verify that the inclusion criteria are respected. The patients will also be evaluated by their physician at the Regional Reference Centres, and the presence of factors known to influence bone mass will be recorded. In particular, attention will be paid to the following factors: respiratory impairment (based on FEV1); number of antibiotic treatments and O2 treatments in the last year; corticosteroid treatment (cumulative dose and duration of treatment); chronic liver disease as defined by: (a) hepatomegaly (confirmed by abnormal ultrasonographic findings such as: increased liver size, nonhomogenous echogenic pattern, irregular surface); (b) abnormal biochemistry (serum aminotransferases, gamma-glutamyl transferase); nutritional condition (Cole index: weight/height); pubertal stage; dietary calcium intake; vitamin supplementation; lung or liver transplantation.

NUMBER OF VISITS PER PATIENT: 5 visits in part 1; 4 in part 2 (baseline visit plus 1 visit every 3 months).

SCHEDULE

STUDY PROTOCOL

The study is divided in 2 parts. Each part will last 1 year for each patient.

PART 1

The first part of the study includes the following steps:
-- baseline = clinical evaluation (1); biochemical tests (2); BMD measurement (3); interview by a dietician to evaluate the patient’s daily calcium intake
-- at 3 months = biochemical tests (2)
-- at 6 months = clinical evaluation (1); biochemical tests (2); BMD measurement (3)
-- at 9 months = biochemical tests (2)
-- at 12 months = clinical evaluation (1); biochemical tests (2); BMD measurement (3)

After baseline evaluation, the daily calcium intake will be optimized, if needed, to the recommended daily allowance (RDA), and all the patients will receive 25-OH vitamin D (calcifediol) orally for the full period of 12 months, at the following dose: 15 μg/day for body weight < 20 kg; 25 μg/day for body weight between 20 and 30 kg; 35 μg/day for body weight > 30 kg.

(1) weight, height, pubertal stage; complications of CF; intercurrent treatment; compliance; dietary questionnaire; physical activity questionnaire.

(2) (local laboratory) serum and urinary calcium, phosphorus, creatinine, magnesium; (central laboratory, at all times) bone markers (bone specific alkaline phosphatase; osteocalcin; telopeptide N-terminal of procollagen I) and 25-OH D; (central laboratory only at baseline, at 6 and at 12 months) iPTH and 1,25(OH)2D; (central laboratory, only at baseline and at 12 months in order to ensure comparable data) serum and urinary calcium, phosphorus, creatinine, magnesium.

(3) BMD will be measured on lumbar spine (L1-L4) at each participating centre using a DXA device. Where possible, BMD will also be measured on total body and at hip.

MONITORING THE SIDE EFFECTS OF 25 OH VITAMIN D

Since 25-OH vitamin D may cause hypercalciuria (urinary calcium/urinary creatinine >0.23 or urinary calcium excretion > 4 mg/kg/day) and/or hypercalcemia (Ca > 10.5 mg/dl), the urinary and plasma calcium will be monitored monthly. Hypercalciuria or hypercalcemia will cause the patient’s drop-out from the study and 25-OH vitamin D will be discontinued. The patient’s data will be evaluated with the procedure of “intention-to-treat”.
**PART 2**

Patients who will show a BMD increase of less than 5% at lumbar spine (L2-L4) after 1 year of adequate dietary calcium intake plus 25-OH vitamin D will be included in Part 2 of the protocol. These patients will be randomly assigned to either placebo or alendronate treatment. The following dosages of alendronate will be used: 5 mg/day, for body weight up to 25 kg; 10 mg/day, for body weight of more than 25 kg. The drug or placebo will be taken “per os” daily, with a glass of oligomineral water: in the morning, at least 45 minutes before breakfast; or in the afternoon, preceded and followed by a fast of two hours or more. The treatment will be continued for 12 months.

**RANDOMIZATION:** the patients will be assigned to either placebo or drug treatment through a centralized balanced randomization procedure controlled by a statistician. For every patient entering Part 2, each centre will communicate the patient code (a progressive number) plus gender and age to the Coordinating Centre, who will respond with the randomization (allocation to treatment arm A or B), and will send the 6-month treatment package for that patient. The placebo and the drug will be identically packaged to respect the double-blind procedure.

The second part of the study includes the following steps:

-- baseline = clinical evaluation (1); biochemical tests (2); BMD measurement (3)  
(Note: The tests at 12 months of Part I will be used as Part 2 baseline tests)

-- at 3 months = biochemical tests (2)

-- at 6 months = clinical evaluation (1); biochemical tests (2); BMD measurement (3)

-- at 9 months = biochemical tests (2)

-- at 12 months = clinical evaluation (1); biochemical tests (2); BMD measurement (3)

(1) (2) (3) see notes to Part 1 above

**MONITORING THE SIDE EFFECTS OF ALENDRONATE**

The following known side effects will be checked monthly: gastric pain, abdominal pain, nausea. Patients will be instructed to refer any other presumable side effect. In the presence of severe treatment-related side effects, the patient will stop the alendronate and drop out from the study. The patient’s data will be evaluated with the procedure of “intention-to-treat”.

**METHODS**

Serum and urinary calcium, creatinine, phosphate, magnesium and serum alkaline phosphatase will be measured by each Centre with standard laboratory methods. These data will be used for the monitoring of the patients. At baseline and at 12 months, serum and urinary calcium, creatinine, phosphate, magnesium will also be measured with standard laboratory methods at the Coordinator Centre, in order to double-check all the results with a single method. 

At the Coordinator Centre, serum bone specific alkaline phosphatase will be measured by immunoenzymatic method (Metra Biosystems Corp.); serum intact parathyroid hormone by IRMA (DiaSorin Corp.); serum osteocalcin by RIA (Nichols Institute Corp.); serum 25-OH D by RIA (DiaSorin Corp.); serum 1,25(OH)2 D by radioreceptor assay (Incstar Corp.); urinary N-terminal telopeptide of procollagen I by RIA (Ostex International Corp.).

BMD (mg/cm2) will be measured at lumbar spine (L2-L4) by dual X-ray absorptiometry (DXA); where possible, also total body and hip BMD will be measured. Bone mineral density (BMD) evaluation is clearly a key point of the study and will be managed with extreme rigor. Of course, as in all other national or international multicentric studies, it is not possible to have patients to travel to a single location to have a DXA exam on a single instrument.

However, since the chief goal of our study is appraising subtle changes in BMD, a strict quality control procedure has been developed and will be applied by all participating centres. Each patient will have all the DXA scans on the same machine. Positioning and scanning patients as well as data analysis will be done at each participating centre according to a standardized protocol. There will be daily instrument calibration at each centre with the local spine phantom, and regular cross-calibrations of all the instruments with a common study phantom circulated among the centres, at least twice a year.

The Coordinator Centre will be responsible for the quality control of all the BMD measurements. A printout and a diskette output of each scan will be sent to the Coordinator Centre, where a trained physician (not involved in the patients’ follow-up) will check all the scans. She will request that those not attaining the desired quality be either performed again in a few days (e.g. for errors in positioning) or reanalyzed (e.g. for incorrect separation of the vertebral bodies).
STATISTICAL ANALYSIS

a. SAMPLE SIZE
Taking BMD as the variable to be studied for the quantitative evaluation of treatment response, and assuming its value to be between 500 and 1200 mg/cm\(^2\) (mean = 800 mg/cm\(^2\)), in order to compute the number of patients required for the second part of the study (alendronate vs placebo), we considered three different scenarios:

1. NULL HYPOTHESIS: average percent BMD increment due to increasing age = 1.25% (average absolute increment 10 mg/cm\(^2\) = m0). ALTERNATIVE HYPOTHESIS: average percent BMD increment for alendronate-treated patients = 5% (40 mg/cm\(^2\) = m1). Assuming an equal variability in both groups, and assuming increments in the alendronate-treated group ranging from 0% to 15% (0 to 120 mg/cm\(^2\)), standard deviation (SD) would be 30 mg/cm\(^2\); to be conservative and considering also measurement errors, SD will be assumed = 35 (s). Two samples, two-sided test, 5% significance level, 90% power: n=29.

2. NULL HYPOTHESIS: average percent BMD increment due to increasing age = 3% (average absolute increment 24 mg/cm\(^2\) = m0). ALTERNATIVE HYPOTHESIS: average percent BMD for alendronate-treated patients = 5% (40 mg/cm\(^2\) = m1); SD 35 (s). Two samples, two-sided test, 5% significance level, 90% power: n=101.

3. NULL HYPOTHESIS: average percent BMD increment due to increasing age = 3% (average absolute increment 24 mg/cm\(^2\) = m0). ALTERNATIVE HYPOTHESIS: average percent BMD increment for alendronate-treated patients = 8% (64 mg/cm\(^2\) = m1); SD 45 (s). Two samples, two-sided test, 5% significance level, 90% power: n=27.

Considering also the placebo-taking subjects, the total number of patients to be included in Part 2 of the protocol (according to the actual mean BMD increment due to alendronate and to the increasing age, and considering also biological variability) will then be 58 in the first scenario, 202 in the second and 54 in the third. Our preliminary data indicate that, after Part 1, approximately 1/3 of the patients will enter Part 2 (i.e. about 1/3 of the patients, notwithstanding adequate calcium intake and 25-OH vitamin D, are expected to have a BMD increase of less than 5%).

The numbers of patients needed to perform our study will thus be:

<table>
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<th>SCENARIO</th>
<th>n in Part 1</th>
<th>n in Part 2</th>
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<tr>
<td>1</td>
<td>180</td>
<td>58</td>
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<tr>
<td>2</td>
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<td>202</td>
</tr>
<tr>
<td>3</td>
<td>165</td>
<td>54</td>
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</table>

According to past experience, scenario 2 is pessimistic. A more realistic one would be a compromise between scenarios 1 and 3. We presume that it will be necessary to enroll approximately 250 patients in Part 1, to allow for some unavoidable losses during the follow-up. The available data from the Milano Centre, where BMD was systematically measured, show that out of 120 patients, 40 patients (33%) would meet the criteria to be enrolled in Part 1. This centre follows approximately 400 patients: it is thus expected to eventually enroll at least 100 patients. This multicentre trial involving 12 centres, following more than 1000 patients, should easily find 250 of them meeting the inclusion criteria. Considering 180 patients in Part 1 and a paired test (two-sided, 5% statistical significance), an actual difference “before/after” of 1% (s=35) or 1.4% (s=45) will be found (power 90%).

b. STATISTICAL METHODS
Student’s t test and appropriate confidence intervals will be used to estimate comparison of the means, after evaluating the validity assumptions. To evaluate the effect of covariates, regression analysis and comparisons for stratified data in value classes will be used, after evaluating the validity assumptions.

c. EVALUATION OF EFFICACY
The efficacy of the treatments (Part 1: calcium plus calcifediol alone; Part 2: placebo versus alendronate) will be evaluated on the basis of BMD increase. A yearly BMD increase of at least 5% at lumbar spine will be considered a positive result.

Those with a BMD increase of less than 5% at lumbar spine will continue with Part 2 (alendronate trial). The results of the trial will be evaluated by comparison of: (1) the mean BMD increases of the two samples (alendronate or placebo); (2) the number of patients with a positive increase in the two samples (alendronate or placebo).

REFERENCES


COORDINATOR’S TEAM AT ISTITUTO AUXOLOGICO ITALIANO - MILANO

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