Construction of a database for the evaluation and the clinical management of patients with breast cancer treated with antiestrogens and/or aromatase inhibitors

Francesca Giusti
Silva Ottanelli
Laura Masi
Antonietta Amedei
Maria Luisa Brandi
Alberto Falchetti

SOD Malattie del Metabolismo Minerale ed Osseo,
AOU Careggi, Florence, Italy

Address for correspondence:
Alberto Falchetti, M.D.
SOD Malattie del Metabolismo Minerale ed Osseo,
AOU Careggi, Florence, Italy
Viale G. Pieraccini, 6 50139 Firenze
Tel. 055-4271502
Fax 055-4271506
E-mail: a.falchetti@dmi.unifi.it

Summary

Breast cancer, mostly exhibiting an hormone-dependent pathogenesis, is a commonly diagnosed cancer in females. It is well known that sex steroids favor the process of carcinogenesis of breast tissue and anti-hormonal therapy of breast cancer aims to decrease the action of estrogens on this tissue. For this purpose, two different compounds are prevalently used: the Selective Estrogen Receptor Modulators, preventing the cancer cell to interact with estrogens, and Aromatase Inhibitors, inhibiting the tissue conversion of androgens into estrogens. Unfortunately, latter treatments negatively impact on bone mass leading to the onset of osteoporosis. For this purpose, we propose to build a database to afford, to store and analyze information about the effects of treatment with Selective Estrogen Receptor Modulators and/or Aromatase Inhibitors on bone metabolism in patients with breast cancer referred to Our Center. We will focus on the possibility of intervening to reduce the negative effects on bone both by the identification of modifiable risk factors and administration of specific therapies, in order to create a therapeutic, diagnostic standard workup for these diseases.

KEY WORDS: breast cancer; anti-hormonal therapy; osteoporosis, fragility fractures; clinical database.

Introduction

Breast cancer (BC) (OMIM #114480) is the most commonly diagnosed cancer among women and in 2008 has been reported to account for 26% of all new cancers (1). In the United States, it has been estimated that the prevalence of BC in the life of a woman is 1:8 (2), representing the second major cause of cancer-related death. Recently, the survival rates have been demonstrated to improve (2, 3).
BCs are mostly hormone-dependent tumors, the cells of which frequently express hormone receptors for estrogens (ER) and progesterone (PgR). These sex steroids initiate and promote the process of carcinogenesis of breast tissue by increasing the rate of cell division and reducing the time available for DNA repair.
The aim of anti-hormonal therapy of BC is to decrease the action of estrogens on breast tissue acting on two possible mechanisms by two different compounds: 1) the Selective Estrogen Receptor Modulators (SERMs) to prevent the cancer cell to use estrogens by interaction with ERs, modulating their response after the SERMs-receptor complex formation; 2) Aromatase Inhibitors (AIs), which inhibit the peripheral conversion of androgens into estrogens, countering the growth of cancer cells and leading to apoptosis.
Recent improvements in screening, diagnosis and treatment of BC resulted in the treatment of 64% of BC cases diagnosed in the early stages of the disease (4). In such affected women, the survival rates of 98% at 5 years have been reported, allowing to continue the treatment for many years (4-6).
Unfortunately, some of these therapeutic agents, the AIs, may lead to other co-morbidities, such as an excessive bone loss that facilitates the onset of osteoporosis (7, 8). Therefore, the complications resulting from these longstanding treatments have to be adequately addressed in this population.

Estrogens and their receptors

Estrogens are a class of sex steroid hormones synthesized starting from cholesterol in ovary, adipose, adrenal and placental tissues (9). 17-β-estradiol (E2) is the most abundant and active natural estrogen, which exerts its effects by binding directly to ERs (10). Its binding to ER induces a conformational change in the structure of the receptor protein, making possible either homodimerization or interaction with molecules acting as co-activators (11, 12). The transcriptional activation of genes occurs through direct interaction of the complex formed by the ligand and by homodimer coactivator proteins with the portion of DNA named estrogen response elements (EREs) located in the promoter region of the gene (11, 13-17). Therefore, ERs are members of a superfamily of inducible nuclear receptors acting as transcription factors mediating the biological effects of the steroid hormone (18). Specifically, these receptors have a conserved structure consisting of five different domains (18-20) (Figure 1).

Estrogen receptors isoforms

Two isoforms of the ERs (ER-α and ER-β) have been described (Figure 1). ER-α is expressed primarily in breast, vagina and uterine tissues, while high levels of ER-β are present in the central nervous, cardiovascular, gastrointestinal, and immune systems, as also in kidneys, lungs and bones (19). Both the isoforms show considerable sequence homology in their functional domains. ER-α and ER-β share 97% homology in the DNA binding domain (DBD) and are identical for 59% in the ligand binding domain (LBD) region (20) (Figure 1). The activity of the activation function 1 (AF-1) is regulated by growth factors that are involved in the Mitogen Activated Protein (MAP)-kinase cell "pathway" (21). The activity of activation function 2 (AF-2) region is regulated through the bin-

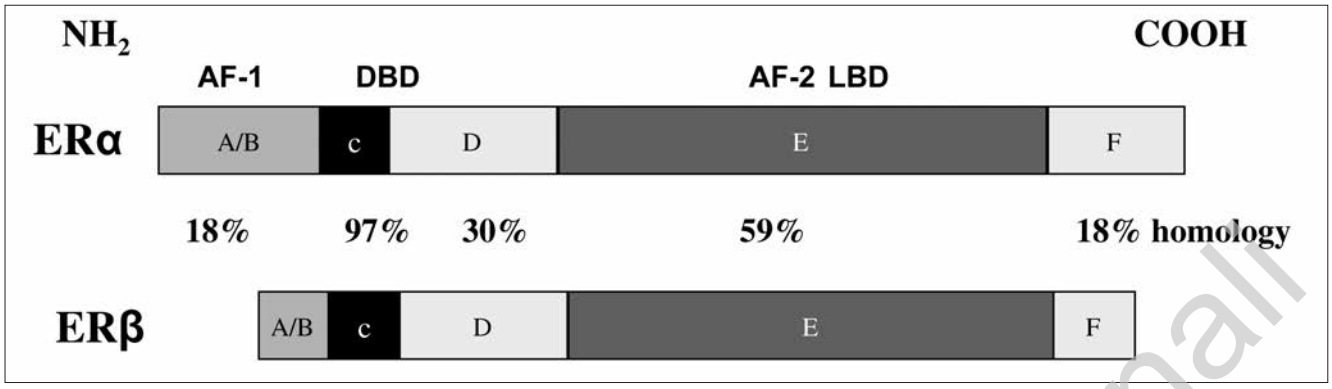

Figure 1 - The amino terminal region, called A/B domain, is the least conserved between the different members of the family of nuclear receptors. It contains a domain called AF-1 (Activation Function 1), which stimulates the transcription of target genes independently of the ligand; the C domain or DNA binding (DNA binding domain, DBD), is the most conserved and determines the specificity of the receptor compared to a class of genes. In fact, different receptors recognize different consensus sequences. The DBD contains two α helix finger-shaped structures, called "zinc finger" in which a Zn^{2+} ion is coordinated by four cysteines. The first one is the P-box (proximal box) that allows to recognize a specific DNA sequence and the second one is the D-box (distal box), which is involved in DNA dimerization; the D domain is a "hinge" region. It connects the C domain to the E domain and it is the binding site to heat shock proteins (HSP) 90 chaperonine. It also contains nuclear localization sequences (Nuclear Localization Signal, NLS); the E region, besides being the ligand binding domain (Ligand binding domain, LBD), contains a domain for receptor dimerization and mediates the interaction with HSP. At the LBD level localizes the AF-2 (Activation Function 2) domain, involved in the ligand-dependent transcription. Finally, inside the E domain an NLS is contained; the F carboxyl-terminal region is poorly preserved and is only present in some nuclear receptors, including ERs.

ding to ligand that alters the structure of the LBD; thus, it creates a modification of number of 12α helices (H1-H12), conserved structure, which induces a conformational change within the AF-2 functional domain (22, 23). When the ligand interacting with LBD is an agonist molecule, such as the endogenous 17-β-estradiol, or synthetic diethylstilbestrol (DES) (20), the H12 helix changes the spatial arrangement, positioning above the hydrophobic pocket, stabilizing the interactions between receptor and ligand and forming an amino acid surface important to the recruitment and interaction with co-activators nuclear proteins (23).
Instead, when the ligand is tamoxifen, which has a bulky side chain extending outside LBD, the H12 helix undergoes modification that does not move the binding pocket, consequently not leading to expose the binding site of co-activators. Such a mechanism seems to explain the antagonist activity of tamoxifen (24).
Many proteins interact with ERs showing a preference for one of the two transactivation domains, AF-1 or AF-2, so as to lead a conformational change of the receptor leading to activation or repression of the transcription of the responsive genes (18, 19 25-27). Other proteins are also able to bind to the ER in a ligand-dependent manner. They are referred to as transcriptional co-activators since they amplify the transcriptional activation due to ER-α dimerization, as also other nuclear receptors (28, 29). The transcriptional co-activators Steroid Receptor Coactivator-1 (SRC-1) and Glutamate Receptor Interacting Protein-1 (GRIP-1) bind to the LBD region of ER-α through the recognition of the small amino acid sequence LXXLL (where L is Leucine and X any amino acid), namely the NR-box (30, 31).
These mechanisms of interaction between estrogens and ERs represent the molecular basis for the development and functioning of the female reproductive system, the proliferation and differentiation of the mammary epithelium (32), but also for the protective effects on the cardiovascular system, the quality of bone structure (maintenance of bone density and reduction of the risk of fractures) and to regulate body temperature by influencing the brain centers controlling this function (33-35). At the time of menopause, the rapidly declining estrogen levels triggers a series of changes such as the increase of LDL cholesterol, the increased incidence of heart diseases, the decrease in bone density favoring the onset of osteoporosis (36).
The understanding of these mechanisms of interaction have led to the development of the hormone replacement therapy (HRT) that provides protection to the onset of some of the diseases mentioned above (36), but at the same time explains the correlation between the intake of estrogens in post menopausal osteoporosis and the risk of developing BC and uterus cancer (37-39).

BC as an hormone-dependent disease

The BC represents the first recognized hormone-dependent disease by the British physician George Beatson. In 1896, he showed that the total removal of the ovaries (oophorectomy) induced the regression of BC in pre-menopausal women (40). Lately, further studies showed that estrogens are among the main factors determining the onset and/or progression of BC, because they stimulate the proliferation of both healthy and cancer cells through the induction of proteins involved in the nucleic acids synthesis, thus resulting in the activation of genes that regulate cell division (41, 42).
These proteins, specific estrogen metabolites called catechol-estrogen 3,4-quinones (CE-3,4-Q), impair the function of the enzymes involved in transcription and/or replication of DNA, allowing the formation of DNA mutations accounting for the progression of healthy cell toward the acquisition of an hyperplastic and/or tumor phenotype (43, 44).
Therefore, it is important to consider that those compounds, used in the treatment of BC, are able to interact and modulate the transcriptional activity of ER-α in relation to estrogen binding.

SERMs

The ability to search for antagonists interacting with ERs has pioneered the development of compounds that modulate the activity of ER-α and ER-β.
The ligands obtained by synthesis are referred to as SERMs (45). This term refers to their ability to act as estrogen agonists in certain tissues (bone, liver and cardiovascular system) and as antagonists in other body tissues (mammary glands and brain), while in uterus may play both as agonists and antagonists (46, 47). Currently, there are several categories of SERMs, divided into four

generations, developed to make an improvement in benefits by reducing the side effects mostly associated with first generation of SERMs (20).
The first generation belongs to the class of chemical triphenylethylene derivatives and includes the prototype of SERMs, tamoxifen and its derivatives: Toremifene, Droloxifene and Idoxifene (48).
The second generation of SERMs originates from benzothiophene and includes raloxifene, the main compound, from which some derivatives were lately obtained (48).
The third generation has as a "scaffold" reference the benzothiophene and it includes Arzoxifene, while the fourth generation of SERMs, including Acolbifene, consists of benzopyran derivatives (48).
There are at least four classes of electrophilic metabolites that may induce SERMs activation: carbocations, metid chinons, metid dichinons o quinones. Triphenylethylene derivatives, such as tamoxifen, are hydroxylated in position α by cytochrome P450 (3A, 2D6, 2C9, 1A1, 1A2 and 1B1) (49).

1st generation SERMs

Tamoxifen (Nolvadex) was, in 1970, the first orally administered drug in patients with metastatic BC (50). The first clinical trial was published in 1971 (51). Later, in the U.S. it has been studied how tamoxifen could have anticancer properties, when administered as an adjuvant, in early stage disease; the results indicated that the incidence of BC was significantly reduced with a prolongation of the survival and disease-free period after the first five years of treatment (52). Subsequent studies have suggested that tamoxifen had beneficial effects on both the reduction of invasive BC, in patients with in situ ductal carcinoma (ISDC) (53), and prevention of BC. It was resulted important in patients at increased risk of developing BC due to the age or the presence of a positive family history or a personal history of an in situ lobular carcinoma (54).
According to what above reported, tamoxifen has been used in clinical practice as an adjuvant therapy in women with tumors positive for estrogen receptor (ER^+) after surgery and/or chemotherapy.
However, significant side effects, such as increased endometrial neoplastic, thrombosis and embolic phenomena, have been reported (55, 56). Further studies have shown that a prolonged use of tamoxifen may lead to the occurrence of liver tumors, such as hepatocellular carcinoma (57), exhibiting frequent and specific mutations in the *p53* tumor suppressor gene (58) and occurrence of hormone-dependent BC in rats (59). Considering all these factors, the existence of tissue-specific target genes regulation by SERMs may exist (17). It has been observed that agonist action of tamoxifen in uterine tissue could be specifically attributed to the SRC-1 coactivator, expressed here at high concentrations and much lower in other areas of the body, such as breast epithelium, in which tamoxifen perform its function as an antagonist (17). Consequently, this estrogenic activity could represent the largest contribution to the carcinogenic effects of the drug at the endometrial level (17).
The Droloxifene, with a hydroxyl in position 3, exhibits an anti-estrogenic activity *in vitro*, equivalent or slightly superior to tamoxifen (60). It has not reported to induce no errors in the DNA and liver tumors in rats (61).
The 4-iodo derivative of tamoxifen, known as Idoxifene, has anti-estrogenic activity fully comparable with other compounds and generating no carcinogenic effects in rats (62).
Toremifene (Fereston) can act as an anti-estrogen in breast tissue and has also positive effects on bone density; however, it exhibits an agonist effect on endometrial cells, even fewer than reported for tamoxifen (20). Indeed, the occurrence index of the endometrial cancer in patients treated with toremifene is 1.4 versus 2.0, reported in patients undergoing treatment with tamoxifen (63). Toremifene is used only in women with advanced BC (20).

2nd generation SERMs

Great enthusiasm was created by the discovery of benzothiophene derivatives since these compounds have no estrogenic activity in the uterus whereas appear as powerful anti-estrogens in breast tissue (64, 48, 49).
Raloxifene (Evista), when used as a chemo preventive medication, reduces the significant risk of developing BC in postmenopausal women with fewer side effects of tamoxifen (65). However, it is not used as chemotherapy in the BC because it has a lower efficacy than tamoxifen in advanced stages of disease (66).

3rd generation SERMs

A member of this family is the Arzoxifene that appears to be a chemotherapic agent with fewer side effects than raloxifene (67, 68). It acts as an antagonist in the uterus (69).
Arzoxifene, and its metabolite DMA, shows high binding affinity to ERs and high capacity to inhibit the estrogen-dependent growth of MCF-7 cell line (70, 71); in fact, assays on this cell line show that this drug has a greater capacity than tamoxifen to inhibit the tumor growth (70).

4th generation SERMs

The compounds belonging to this family are benzopyran derivatives and the best one known is the Acolbifene (EM-652).
This drug is a powerful anti-estrogen molecule, able to inhibit both ER-α and ER-β (71) signaling pathways and consequently the proliferation of cell lines derived from BC and cervical cancer (72-74).

Current perspectives

Currently, the standard postoperative adjuvant therapy in ER^+BC, is represented by tamoxifen for a total of five years of treatment. This therapy allows a reduction in overall mortality with particularly important effects in patients exhibiting the involvement of the axillary lymph nodes. However, the increased risk of developing uterine cancer and thromboembolic phenomena, connected with the use of tamoxifen (58, 59), has prompted researchers to develop new therapeutical strategies leading to the development of new compounds: the AIs.

AIs

The action of these compounds consists of the inhibition of the metabolic pathways leading to the biosynthesis of estrogens in cancer cells. In particular, aromatase enzyme belongs to the P450 cytochrome family, responsible for the synthesis of estrogens starting from androgen precursors and in particular the formation of estrone from androstenedione and estradiol from testosterone (75). This enzyme is present in granulosa cells of ovarian follicles, subcutaneous fat, liver and muscle. At menopause, estrogen production is mainly due to the subcutaneous fat aromatase activity. In fact, there is a direct correlation between "body mass index" and estrogen circulating levels in postmenopausal women (76).
Recent studies have identified the BC tissue as an important site for estrogen production and approximately 2/3 of BC exhibit aromatase activity and synthesize significant amounts of biologically active estrogens, so as to provide a concentration of estradiol in tumor tissue 10 times superior to the plasma values (77).
The total estrogen suppression in postmenopausal women may be reach by the inhibition or inactivation of the aromatase enzyme by AIs. These compounds have a total anti-estrogenic action, lacking of the partial agonistic activity of tamoxifen that allows the latter to have a positive effect on bone and a negative effect on the risk of uterine cancer and venous thrombo-embolism.
The AIs are classified into type 1 inhibitors, or steroid enzymatic inactivating drugs (steroids analogues of Androstenedione irre-

versibly binding to the same site of the aromatase), and type 2 enzyme inhibitors, or nonsteroid enzymatic inactivating drugs (nonsteroid substances reversibly binding to the heme group of the aromatase enzyme) (78).
Three generations of these compounds are known:

1st generation AIs
The first AI used in the clinical practice was the aminoglutethimide, initially used as an anticonvulsant drug, followed by testonolactone not proved to be a potent inhibitor. The use of aminoglutethimide for the treatment of BC has been abandoned because of the complete inhibition on adrenal steroidogenesis, determining a "chemical adrenalectomy". In fact, it accelerates the metabolism of the estrogen sulfate, resulting in lower free plasma and urinary estrogen levels (79), and induces metabolic enzymes mediating and inhibiting the liver enzymes controlling the synthesis of cortisol, aldosterone, thyroxin, and aromatase itself (79). Therefore, administration of aminoglutethimide must be accompanied by administration of glucocorticoids, hydrocortisone, and, in some patients, thyroxin (79).

2nd generation AIs
Fradozolo and formestano belong to this class of inhibitors. The fradozolo is a fairly potent inhibitor of aromatase and shows a significant reduction in toxicity when compared to aminoglutethimide. The main inhibitor of this class is represented by formestano, a structural analogue of androstenedione which shows high specificity for the enzyme, belonging to the type 1 inhibitors class (enzymatic inactivators).
This drug has a significant clinical efficacy, whose limit is represented mainly by the route of administration (intramuscular injection).

3rd generation AIs
The third-generation AIs is represented by anastrozole (Arimidex), letrozole (Femara) and exemestane (Aromasin). In pre-clinical studies, these new compounds have shown that they: a) do not affect adrenal steroidogenesis, since they do not change the basal levels of cortisol and aldosterone; b) have a high pharmacological power (greater three orders of magnitude than aminoglutethimide) associated with a good tolerability; and c) can be administered orally, making these drugs very handy and suitable for a prolonged treatment (77, 78).
Letrozole and anastrozole are type 2 AIs (non-steroidal inhibitors) with a plasma half-life of approximately 48 hours (77).
On the contrary, exemestane is a type 1 AI (steroid activator) with a plasma half-life of approximately 27 hours (77).

Contraindications to the use of AIs

The use of AIs is contraindicated in: 1) pre-menopausal patients: AIs induce an increased secretion of gonadotropins, because of the reduced feedback of estrogen at hypothalamus and pituitary level. In some animal studies, the AIs in premenopausal subjects determine an increase in size and weight of the ovaries (78); 2) women with negative hormone receptor BC since they are not usually responsive to hormonal treatment (78).

Use of AIs in BC

The treatment of women with ER$^+$BC aims to induce deep hypoestrogenism. While in the past the menopause was often induced by surgery, currently the pharmacological castration is the preferred choice. In premenopausal women, the treatment consists of a gonadotropins agonist (GnRH) combined with AIs, whereas in postmenopausal women only AIs are used.

1. AIs as adjuvant treatment in post-surgery for BC
Several clinical trials have begun to test the possible role of third-generation AIs as an adjuvant treatment of BC in postmenopausal women. The first and the most important of these trials is represented by the ATAC (Arimidex and Tamoxifen Alone or in Combination) trial conducted on 9366 patients (80).
After a median follow-up of approximately 33 months, early results showed a small reduction in tumor recurrence rates (87% vs. 89%) in women taking anastrozole compared with those enrolled in the tamoxifen group.
Subsequently, the analysis of the data collected after 4 years of therapy confirmed this behavior. In fact, it has been reported both a longer disease-free survival in 86.9% of patients treated with anastrozole compared with 84.5% of those treated with tamoxifen, as also a reduction in drug-induced side effects in the anastrozole group. The tamoxifen-anastrozole association does not appear to offer additional benefits to the individually use of such compounds. The ATAC (80) also revealed a lower incidence of contralateral occurrence of BC in patients treated with anastrozole compared with those treated with tamoxifen (0.3% vs. 1%). Although adjuvant therapy with tamoxifen remains the standard treatment in ER$^+$BC patients, treatment with AIs may be based on the results obtained in more recent clinical trials, representing a valid alternative in women with high thromboembolic risk or low tolerance to tamoxifen. Recently, anastrozole was approved for the adjuvant treatment of early ER$^+$BC in postmenopausal patients, especially when tamoxifen was contraindicated (81-84).

2. Use of AIs as neoadjuvant treatment of locally advanced BC
The reduction of the tumor mass, before surgery, through the use of endocrine therapy is an attractive option. Some randomized clinical trials on postmenopausal women with ER$^+$BC, larger than 3 cm. in diameter, showed that administration for a few months of anastrozole, letrozole or exemestane was able to determine a higher reduction of the tumor volume than tamoxifen (allowing in most cases the use of a conservative surgical therapy) (85).

3. Use of AIs in the treatment of metastatic BC
Clinical double-blind multicentre studies have shown that AIs of the third generation (particularly letrozole) are superior to tamoxifen as a first line endocrine treatment of advanced ER$^+$BC (85), because these compounds are able to determine a greater tumor reduction and disease-free period (85). In addition, third generation AIs have also proven to be superior to megestrol acetate as a second line endocrine therapy of advanced BC with a lower incidence of side effects.

4. Use of AIs in the preventive treatment of BC
Preliminary results of the ATAC study suggest that AIs, due both to their anti-estrogenic and inhibition of the development of BCs, may have an important role as a preventive drug treatment of BC (80). As mentioned previously, this important clinical trial showed a lower incidence of contralateral BC in women in the arm with anastrozole adjuvant therapy compared with those treated with tamoxifen (0.3% vs. 1%), after a follow-up of about 33 months (80). Unfortunately, even if the preventive efficacy of AIs appears to be superior to that one of tamoxifen, further clinical studies are needed to define their potential use in chemoprevention in women at high risk for BC.

Effects of hormonal therapy on bone mineral density (BMD)

SERMs

Tamoxifen
Tamoxifen binds to both ER-α and ER-β and has a partial ago-

nist effect on bone. *In vitro* and *in vivo* studies on ovariectomized rats have suggested that tamoxifen has effects similar to estrogen in both trabecular and cortical bone (81, 82). These results were further developed through studies in humans by histomorphometric analysis of iliac crest bone biopsies. Women affected by BC treated with tamoxifen were found to have static indices of bone remodeling similarly to women with BC who did not receive tamoxifen (83).

The effect of tamoxifen on bone density or fracture risk is different depending on the premenopausal or postmenopausal status and this may be due to its action as a partial ER agonist. High risk women, treated at premenopause with tamoxifen for 3 years, showed a slight decreases of lumbar spine BMD (-1.44% per year) and a significant gain compared to the modest gain observed in the placebo group (0,24% per year, p <0.001), while small changes in hip BMD occurred in both groups (84).

In contrast, postmenopausal women treated with tamoxifen showed a slight increase in lumbar spine and hip BMD (+1.17% per year and +1.71% per year, respectively) compared to placebo group (84).

Some authors have supported the interaction between menstrual status and BMD response to tamoxifen (85). Patients who developed amenorrhoea induced by chemotherapy had a BMD lower than those who continued to menstruate or without tamoxifen administration. However, in women who continued to menstruate, the use of tamoxifen led to a BMD loss (-4.6% at the spine) in comparison to a modest increase in the women's group not treated with tamoxifen. Among women who developed amenorrhoea, the use of tamoxifen was associated with attenuation of lumbar spine BMD loss (-6.8%) versus the ones not treated with tamoxifen (-9.5%). These findings suggests that tamoxifen has an effect on BMD related to the estrogen levels in premenopausal women. Small decreases in BMD have been reported even with raloxifene treatment (86). It is unclear whether these small effects on BMD, due to the use of SERMs in premenopausal women, result in changes of the relative risk of fractures. The P-1 NSABP Study [National Surgical Adjuvant Breast and Bowel Project Cancer Prevention Trial (P-1)] has reported a decrease in the number of osteoporotic fractures in premenopausal women at high risk for the BC treated with tamoxifen for five years compared to placebo (57). Postmenopausal women treated with tamoxifen have a small increase in spine BMD, which is evident in the early months of the trial and then tends to stabilize (87-90).

In a Danish study, in which postmenopausal women at high risk of BC were treated with local radiotherapy, with or without tamoxifen in the following year, a higher number of hip fractures in patients treated with tamoxifen compared to the control group has been reported (91).

In summary, the use of tamoxifen is associated with a modest effect on BMD in postmenopausal women and a small decrease in BMD in premenopausal women.

Raloxifene

A double-blind study, which involved 601 healthy women (aged between 45 and 60 years) entered into menopause by 2-8 years, randomized to raloxifene (30 mg, 60 mg, 150 mg/day) or placebo, showed, in patients taking raloxifene, a significant increase of 2-3% at both spine and proximal femur BMD compared to placebo. By examining the biopsy specimens of bone tissue, the biomechanical and histomorphometric features of bone were normal and in particular there were no signs of altered mineralization nor bone marrow fibrosis or presence of abnormal lamellar bone (92). The anti-fracture efficacy of raloxifene was assessed, for a period of three years, in the MORE study (Multiple Outcomes of Raloxifene Evaluation), a double-blind study performed on 7.705 postmenopausal women with osteoporosis, with or without the presence of fractures at the beginning of treatment.

After 36 months of treatment, women taking raloxifene (60 mg/die) showed, in comparison with patients on placebo, an increase of 2-3% of BMD at all the skeletal sites examined. Moreover, patients treated with raloxifene showed also a significant reduction in the incidence of new vertebral fractures compared with placebo group (35% for the group of women with fractures before therapy, and 50% for those without fractures at baseline) (93, 94). However, raloxifene was associated with an increased risk of thrombo-embolic events for which it is not recommended in patients who complain or at high risk of venous thrombosis (95).

AIs

At menopause, serum levels of estrogens decrease by about 90% (96) and this leads to an increased bone turnover and a net bone loss, which can take 5-10 years to reach a 30% of trabecular bone loss and 10% cortical bone loss that determine an increase of the fracture risk (97).

The use at post-menopause of anastrozole, letrozole and exemestane lower estrogen serum levels of 81-94%, 88-98% and 52-72% (98), respectively.

Observational studies have found an increased bone loss and fractures rates in women treated with AIs. In these women, compared to those treated with other drugs, a retrospective cohort study on 12.368 patients with BC, has documented significantly higher rates of reduction in bone mass (respectively, 8.7% versus 7.1%) and fracture (respectively, 13.5% versus 10.3%) (99).

A study on 1043 women with BC found that patients treated with AIs had a 2.5 times higher probability of experiencing fractures compared with those treated with tamoxifen (100).

Anastrozole

Evidence of increased bone loss during treatment with anastrozole were evidenced in several studies. The ATAC (Arimidex, Tamoxifen, Alone or in Combination) randomized 6241 postmenopausal women with ER^+BC treated for 5 years with anastrozole or tamoxifen. After 68 months of follow-up a significantly higher incidence of fractures was reported in the anastrozole-treated group versus tamoxifen (respectively, 11% versus 7.7%) (101). The results, updated after a 100 months follow-up period, described an annual rate of fractures higher in the anastrozole than in tamoxifen arm (respectively, 2.93% versus 1.90%), with similar annual rates, after completion of therapy, between the two arms (respectively, 1.56% to 1.51%) (102).

In ABCSG (Austrian Breast Cancer Study Group) 8 and ARNO (Arimidex/Nolvadex) 95 studies randomized 3224 women with BC treated with tamoxifen for 2 years and then undergone three years of treatment with anastrozole or continuously for 3 more years with tamoxifen. After 28 months of follow-up, the combined analysis of the two studies described a small, but significant, increase in fractures rate in women who were switched to anastrozole with respect to those who continued therapy with tamoxifen (respectively, 2.1% to 1%) (103).

A sub-protocol of the ATAC study has evaluated 308 patients with BC for two years, reporting the association between anastrozole and BMD loss, whereas tamoxifen led to a modest increase in both vertebral (respectively, -4.0% to -1.9%) and femoral neck (respectively, -3.2% to -1.2%) BMD (104). Preliminary results at 5 years confirmed the significant loss of BMD with anastrozole, although this loss seemed to slow down after 2 years (105).

In a prospective cohort study on 103 postmenopausal women with BC compared with 114 healthy controls revealed a significant reduction at vertebral and femoral neck BMD after 1 year of treatment with anastrozole (104). However, not even after 30 months of Arimidex/Nolvadex 95 follow-up study (979 randomized women) (106), or after 36 months of follow-up in the Italian Tamoxifen Arimidex trial (448 patients) (107), a significant increase in the incidence of fractures, following treatment with tamoxifen to anastrozole, was revealed.

The inconsistent association between the use of anastrozole and

fracture incidence in different studies is likely to be multifactorial in origin, and includes: 1) the possible effect of tamoxifen previously taken; 2) studies without statistical power sufficient to identify the risk of fracture; 3) different age groups; 4) different rates of baseline BMD and bone mass reduction (108).

Letrozole

Two controlled trials on adjuvant therapy with letrozole have documented conflicting results about the incidence of the fractures rate. The Breast International Group 1-98 study, four arms, has compared 5 years of treatment with letrozole monotherapy, letrozole followed by tamoxifen, tamoxifen alone and tamoxifen followed by letrozole. A comparison between the arms initially provided with letrozole or tamoxifen (4933 randomized patients) showed a significant increase in fractures with letrozole after 51 months of follow-up (respectively, 8.6% versus 5.8%) (109).

However, the MA.17 study, letrozole or placebo after 5 years of tamoxifen (5187 randomized women), has documented a not significant increase in fracture rate with letrozole at 30 months of follow-up (respectively, 5.3% versus 4.6%) (110).

A supporting study to MA.17, which evaluated 226 patients, found a significantly greater loss of BMD with letrozole versus placebo after 2 years at lumbar spine (respectively, -5.4% to -0.7%) and neck total hip (respectively, -3.6% to -0.7%) levels (111). The not significant difference in rate fractures of MA.17 study could be due to inequalities in the Breast International Group study control arm, which provided a substance with known protective effect on bone, in addition to a relatively short follow-up period. No studies, however, had sufficient statistical power to assess the incidence of fractures (108).

Exemestane

Exemestane, irreversibly inhibiting the enzyme aromatase, has raised particular concern about its effects on bone metabolism. Animal studies have documented the possibility that the molecule may have androgenic properties that may decrease the degree of reduction of bone mass (112, 113).

Intergroup Exemestane Study, including 4724 postmenopausal women, provided an initial treatment for 2 or 3 years with tamoxifen and then switched to or a treatment with exemestane or staying on tamoxifen. After an average of about 55.7 months of follow-up, there was a significant increase in the incidence of fractures in patients treated with tamoxifen versus exemestane (respectively, 7.0% versus 4.9%) (114).

A bone sub-protocol concerning 206 patients, found a significant reduction in BMD with exemestane after 6 months (-2.7% at the lumbar spine and -1.4% at the femoral neck), which is then gradually slowed. Women who continued taking tamoxifen did not show any significant change in BMD (115).

Tamoxifen Exemestane Adjuvant Multinational study confirmed a significant reduction of lumbar spine BMD after 1 year of therapy with exemestane versus tamoxifen (116).

A significant loss of BMD, as a result of switching from tamoxifen to exemestane, was also observed in a small study on 70 postmenopausal women with BC (117).

A randomized placebo-controlled trial, lasting two years, on exemestane in 147 postmenopausal women with early stage BC, has documented a significant increase in BMD reduction by exemestane in the femoral neck, with no difference at the lumbar level (118). The evaluation of patients within 1 year after completion of therapy showed a stabilization, without further reductions in BMD in the exemestane arm (118).

After reviewing the literature, we can confirm that there is considerable evidence of an association between both an increased loss of bone mass and fracture incidence in women treated with AIs compared with those taking tamoxifen or placebo, but it is not clear whether differences between different AIs exist in relation to the degree of bone loss.

Direct comparison studies, currently underway, including the MA.27 (exemestane to anastrozole) and Femara Versus Anastrozole Clinical Evaluation (letrozole to anastrozole), will provide comparisons for the loss of bone mass, and other outcomes (108).

Since most of the comparison was made with tamoxifen, which guarantees a certain degree of bone protection, the level of reduction in BMD observed with AIs may appear larger, lacking of a control group treated with placebo (108).

Effects of therapy with AIs and/or SERMs on bone turnover markers

Estrogens play an important role in maintaining the balance of bone metabolism (119).

At menopause, the decline in blood levels of estradiol leads to a significant increase in bone resorption, reflected by the increase of serum bone resorption markers, such as C-telopeptide (CTX) and N-telopeptide (NTX), and the decrease of bone formation markers, such as N-terminal propeptide of procollagen type I (PinP), osteocalcin (OC), bone alkaline phosphatase (bAP) and parathyroid hormone (PTH) (120).

In ER^+BC women, undergoing treatment with AIs, a significant higher than expected increase in bone turnover, with respect their postmenopausal status, has been described. Markers of bone resorption were increased, while the bone formation ones were found to be either decreased or increased (104, 121).

In a previous study on postmenopausal women, treatment with exemestane showed a profile of action on bone metabolism slightly different from the one seen in therapy with non-steroidal AIs (121). Indeed, treatment with exemestane led to a significant increase in bone formation markers, while anastrozole or letrozole did not (122).

Unlike in postmenopausal women with ER^+BC, tamoxifen leads to a normalization of bone markers, as demonstrated by a decrease in bone resorption and formation (104). Several studies have reported that tamoxifen has some beneficial effects on bone metabolism and on the risk of fractures in postmenopausal women (89, 123-125). However, the results showed that these positive effects do not last once the treatment with tamoxifen is stopped.

The study by McCaig et al. showed that in patients treated with AIs there is an increase of bone turnover behind the loss of bone mass. Patients, previously receiving tamoxifen, had a significantly greater increase in bone turnover markers, such PinP, CTX, NTX, bAP and PTH compared to patients not receiving tamoxifen (126).

Even the data from the ATAC study showed that bone resorption and formation were suppressed, respectively of 30 and 15%, in patients treated with tamoxifen compared to the untreated population (104).

In BC patients, initial treatment with tamoxifen and AIs thereafter, a different effect of AIs on bone metabolism was noted compared to that one obtained on women not previously treated with SERMs.

A recent study showed that a previous tamoxifen treatment deeply increases the effects of AIs on bone metabolism, especially in the transition from suspension of tamoxifen at the beginning of AIs therapy. These results are similar to those observed in the Intergroup Exemestane Study (IES) in patients treated with tamoxifen for 2-3 years and then switched to exemestane, in which, within 6 months, there was a significant decrease in BMD from baseline at both lumbar spine and hip levels, 2.7% and 1.4% respectively (127).

Therefore, any benefit that therapy with tamoxifen had produced on bone density was lost after stopping this treatment and beginning the one with AIs.

Therefore McCaig et al. concluded that in patients receiving anastrozole or letrozole after tamoxifen therapy the monitoring of bone

metabolism, as performed in patients starting first-line treatment with anastrozole or letrozole (126), is necessary. It has been noted that the AIs have been associated with an accelerated bone loss, whereas tamoxifen has been shown to offer some protection against bone loss in postmenopausal women (109, 114, 128). However, we have to remember that the AIs therapies have demonstrated a superior efficacy, compared to tamoxifen, in terms of disease-free survival and, in some cases, of overall survival. The IES study, reported that women with ER^+BC treated with exemestane had a reduced risk of death by 17% compared to those treated with tamoxifen (128).

Effect of therapy with bisphosphonate on bone loss induced by hormonal treatment of BC

Bisphosphonates are used in the treatment of women with BC because they have no interaction with ERs or PgRs and because they have demonstrated to increase BMD in postmenopausal women (129).
Several clinical studies have evaluated the efficacy of bisphosphonates in preventing the loss of bone mass in BC. Preliminary data of the study with anastrozole and risedronate and of the ARIBON study, in which women with osteopenia treated with AI were randomized to treatment with risedronate (administered at doses of 35 mg weekly) or ibandronate (dose 150 mg monthly), documented significant reductions in BMD loss after 1 year of bisphosphonate therapy (130-132).
Small studies have shown protective effects with risedronate, pamidronate and zoledronate, in similar patients (133-136). In recent years, the ABCSG assessed the zoledronate in pre-menopausal women randomized to ovarian ablation with goserelin, LH-RH (LH-releasing hormone) agonist, resulting in a reversible ovarian suppression, plus tamoxifen or anastrozole (ABCSG-12). Among the 401 patients enrolled in the bone sub-protocol, those treated with zoledronate showed stable values of BMD, whereas there was a significant reduction of this parameter in those who received ablative endocrine therapy alone (137).
Preliminary results at five years, after 24 months from the end of treatment, indicate that these women continue to experience a loss of bone mass compared to those treated with bisphosphonates (respectively, -6.3% versus -4.0%) (138).
Some recent studies have evaluated the preventive role of bisphosphonates in postmenopausal women treated with AIs and the largest of these trials, called Zometa-Femara Adjuvant Synergy Trials (Z-FAST [United States]/ZO-FAST [Europe]), analyzed the effectiveness of intravenous zoledronate, at the dose of 4 mg every 6 months, in women treated with adjuvant therapy with letrozole and baseline T-score $\geq$-2.0. In a treatment arm, the zoledronate was started simultaneously with letrozole, while in another arm it has been postponed until the recording of a reduction in BMD. The one year results of the Z-FAST study documented an average increase of 1.9% in lumbar BMD from baseline in the arm of early treatment with zoledronic acid, versus an average reduction of 2.4% with the delayed administration: total difference of 4.4% (139). A 36 months of follow-up showed that the absolute difference between the two arms, in lumbar spine BMD, had increased to 6.7%, with a greater number of fractures in the delayed than that simultaneously treatment arm (respectively, 6.3% versus 5.6%), although the study had not sufficient power to detect significantly differences in fracture rates (140).
Results at 1 year of ZO-FAST are similar with an overall difference of 5.7% between the study arms in favor of an early administration (141).
After 24 months of follow-up, the results still show a significant difference in BMD in favor of the early treatment with zoledronate (142).
These data are also confirmed by a subsequent study that evaluated the ability of zoledronic acid to preserve BMD when started simultaneously with letrozole in patients with BC and previously treated with tamoxifen (143).
A recent study confirms that post-menopausal women with a T-score <-2.0 are at increased risk of fracture. Treatment with AIs has been shown to improve disease-free survival in women with ER^+BC, but it was also associated with an increased bone loss and increased incidence of fractures than other therapies. This study has shown that concomitant therapy with intravenous zoledronic acid is associated with improvement and/or preservation of BMD in these women (144).

Aim of this project

In medicine, the need of information, accurate from an analytical point of view, credible from a clinical point of view, valid from a statistical point of view, led to the creation of systems capable of providing not only a valid epidemiological support for the planning and management of health interventions (such as activities involving the use of drugs in prevention), but also estimates of incidence, prevalence and mortality of the affected population.
Preventive activities, that we have to currently play in the health's field, should avoid the occurrence of a future adverse event, or should delay its onset. This will allow us to save on future health's costs. Osteopenia and/or osteoporosis, and consequently fragility fractures occurrence, in patients with BC are potentially preventable conditions and therefore careful basal assessment, followed by a continuous monitoring of therapeutic interventions, may prevent or reduce the risk of adverse events, such as the fragility fractures.
So, with this project, we propose to build a clinical database to afford, to store and analyze, on a continuative and systematic manner, information about the effects of treatment with SERMs and/or AIs on bone metabolism in patients with BC referred to Our Center. We will focus on the possibility of intervening on slowing the negative effects on bone both by the identification of modifiable risk factors and the use of specific drugs, so that to create, with the information obtained, a therapeutic, diagnostic standard workup for these diseases.

Construction of the database and discussion

The achievements of these objectives requires analysis of three critical dimensions of the data collection (material): 1) the extension of the population must be designed to get a database that is sufficiently representative of the population concerned; 2) the depth and the extent of data; 3) the time must be aimed to create a database with a time extension sufficient for a reliable assessment of the diagnostic and therapeutic pathways.
Taking into account these considerations, we have built an Excel file (method) to divide the patients into two groups: 1) those treated with tamoxifen and AIs; and 2) those treated with AIs as a first-line therapy.
Then, for each group the following fields for data collection were set:
-1st field: Anamnesis
-2nd field: Oncology
-3rd field: Diagnosis of bone loss
-4th field: Therapy

1st field: Anamnesis
The first area involves the collection of the patient's family and physiological history, from which we extrapolate the data correlated with the development of BC and osteoporosis.
Our attention has focused, in particular, on some aspects of life such as social-reproductive-age, family history of BC and osteo-

porosis, age at menarche, number of pregnancies, breastfeeding, oral contraceptive use and duration of therapy, age at menopause, use and duration of hormone replacement therapy, smoking habits, body mass index (BMI), physical activity, diet intake of dairy products, history of previous fractures, presence of co-morbidities. The risk factors, correlated with both BC and osteoporosis, that we identified and listed below have, as a common denominator, their effect on the level and duration of exposure to endogenous and exogenous estrogens (Table 1):

- **Age:** over 80% of cases of BC and osteoporosis affect women over 50 years (2, 145).
- **Familiarity:** for BC about 10% of women with BC has a family member with BC, especially in cases where juvenile cases are presenting in which some genes, predisposing to the occurrence of this tumor such as *BRCA1* and *BRCA2*, are involved. Mutations of these genes are responsible for 50% of hereditary BC forms (2).

Currently, we know that various factors, both environmental and genetics, contribute to the pathogenesis of osteoporosis. Genetic factors are represented by a pool of genes that regulate the expression of the characteristics associated with the development of the disease (mass and bone microarchitecture) being responsible for 50-80% of the interindividual variability in BMD (146-150). A major contribution to evaluate the influence of environment and genes on phenotypic variability in BMD and the development of osteoporosis have been provided by studies on mono-and dizygotic twins (146, 147, 151-154).

Even studies of family groups have confirmed the existence of such a contribution, showing a correlation between vertebral BMD in mothers and daughters, the BMD of the daughters of osteoporotic women compared with women of that age appears to be reduced and associated with an increased risk of fracture after menopause (151, 152).

- **Age at menarche:** later menarche lesser the BC risk (2), while for osteoporosis a late menarche is associated with reduced bone formation (145).
- **Number of pregnancies** (specifying the earliest age at first pregnancy, the presence of abortion and/or voluntary termination of pregnancy). This period of reduced estrogen production has a protective effect on the development of BC. More numerous are the children greater is the protection. Such a protection seems to be preceded by a short period (several years), immediately after pregnancy, which noted an increase in the risk of BC. Therefore, having children leads to a reduction of long-term risk versus nulliparous (2). After delivery, osteoporosis can be facilitated by a diet poor in calcium during the months of pregnancy, or an inefficient hormonal regulation of calcium metabolism, which creates a negative calcium balance with easier occurrence of gestosis (152).
- **Breastfeeding and its duration:** Breast feeding allows the cell to complete its maturation and makes it more resistant to possible neoplastic transformation (2). Osteoporosis during lactation is linked to an increased need of nutrients like calcium and vitamin D (152).
- **Use of oral contraceptives (OC) and duration:** several studies suggest a slightly increased risk of BC associated with the use of OCs, the risk appears to decrease with age and time extent from the their interruption. In fact, after 10 years from the cessation of OC, the risk of BC returns at the average of the general population (2).
- **Age at menopause**: earlier the menopause lesser the BC. A 10 years anticipation of menopause halve the risk of BC (2). For osteoporosis, it is known that later the menopause higher the estrogen levels that may prevent bone mass loss (145).
- **Hormone replacement therapy (HRT) and its duration:** An increased risk of BC incidence and mortality by the use of HRT in postmenopausal women has been reported. The risk is directly associated with duration of exposure (2). The HRT prevent bone loss at menopause (145).

Table 1 - 1st field: Anamnesis.

ANAMNESIS:
- Risk factors correlated with BC and osteoporosis:
- - Age
- - Familiarity
- - Age at menarche
- - Number of pregnancies
- - Breastfeeding and its duration
- - Use of oral contraceptives (OC) and duration
- - Age at menopause
- - Hormone replacement therapy (HRT) and its duration
- - Smoking habits
- - BMI
- - Physical Activity
- - Recruitment of dairy products
- - Previous fractures
- - Presence of co-morbidity/drugs

- **Smoking habits:** smoking increases the risk of fractures. Combined analysis of studies on 60,000 subjects in Canada, USA, Europe, Australia and Japan have shown that smoking increases the risk of fragility fractures (155). For the BC, this correlation is still controversial, with some studies indicating that smoking leads to an increased incidence of BC (156).
- **BMI:** an increase in BMI in postmenopausal women relates to an increased risk of developing BC due to the production of estrogen by the adipose tissue (2). A low BMI below the value of 19 may predisposes to osteoporosis. In fact, the bone is a dynamic tissue that responds to the load and subjects with a BMD >20 tend to have higher BMD and consequently a more resistant bone structure (155). It is well known that both a low BMI and weight loss are strongly associated with either low bone mass or an increase in fracture risk, while obesity protects against osteoporosis (157).
- **Physical Activity:** Physical activity, through the reduction of body fat, has a protective role against BC either before or after menopause (2). In childhood, physical exercise favors a high peak of bone mass and is recognized as a protective factor against bone stress fracture. In case of high peak, the aging-related bone depletion will be difficult to conduct the subject below the fracture threshold (158). Even in menopausal women physical activity may prevent bone loss. When physical exercise is associated with HRT or calcium supplementation, the effect on bone density is strengthened. Physical activity may help to increase bone density even around 40 years of age, but it has not proved to be effective in reducing fractures in postmenopausal subjects (158). Moreover, exercise helps to reduce the risk of falls and, consequently, the risk of fracture, since it improves the sense of balance, maintaining a close relationship between joint and muscle mass (158).
- **Recruitment of dairy products:** The requirement varies according to age. An inadequate daily calcium intake during the juvenile or in certain stages of life, such as pregnancy and lactation, increases bone resorption, decreases bone formation and reduces skeletal mineralization, thus predisposing to osteoporosis. However, calcium is effective in reducing vertebral and non vertebral fractures only if and when is associated with vitamin D3 (159). Vegetarian people eating raw foods have a low BMD without signs of an increased bone turnover. Given the low calories and protein intake, they usually have a low BMI and low fat content. Such a diet is associated with low bone mass even in the presence of proper vitamin D values (155, 148).
- **Previous fractures:** Multiple studies show that patients with a previous fragility fracture are at a higher relative risk of fracture than subjects who have never experienced fractures (145).
- **Presence of co-morbidity/drugs:** Several studies have evaluated the effects of certain co-morbidities history of BC (such as

type II diabetes or cardiovascular diseases) reporting a negative effect (160). The presence of diseases such as diabetes, hyperthyroidism, hyperparathyroidism, hypercortisolism interfere with calcium metabolism, promoting a secondary osteoporosis, as well as prolonged therapy with corticosteroids (more than three months at a minimum prednisolone-equivalent dose of 7.5 mg/day), thyroxin TSH-suppressive doses, anticonvulsants (145).

2nd field: Oncology

The BC is increasingly early diagnosed, but unfortunately not all diagnoses can be performed at the same stage of disease, so either the patient's health status or the type and grading of tumor may influence the therapeutic choice. Particular importance will be given to the collection of data related to the time between diagnosis of BC and the beginning of hormonal therapy, assessing both whether the therapy is a first-line choice and the presence, in percentage, of ER^+ and PgR^+. Thus, since this database can be used as a source for future studies, we are collecting such information and dividing the patients in different groups, as reported at Table 2.

3rd field: Diagnosis of bone loss

Osteoporosis is a metabolic skeletal disorder characterized by compromised bone strength that predisposes to an increased relative risk of fracture (161). Bone is a dynamic tissue that undergoes, during the life, processes of resorption and formation. In fact, the bone mass of adults will depend both on the peak bone mass reached at young age and the lost due to aging. Peak bone mass is reached around the middle of the third decade. After a plateau period, a period of net bone loss begins (about 0.3-0.5%/year). At menopause, women may lose bone at a 3-5%/year rate. Currently, we know that bone fragility arises from changes in quantity (mass and density), and/or quality (macro and microarchitecture, material properties) (161). The W. H. O. identifies as the gold standard diagnostic criterion for osteoporosis a densitometric reduction in bone mass below 2.5 standard deviations from peak bone mass (T-score <-2.5), assessed by double X-rays bone densitometry (DXA) technique (162). However, osteoporosis is poorly symptomatic and with important medical and social complications, i.e. fragility fractures, whose prevalence and incidence increases exponentially in proportion with the reduction of bone mass (163).
The peripheral quantitative computerized tomography (pQCT) allows to obtain a separate bone mass determination at both cortical and trabecular component, including an accurate assessment of the geometric characteristics of bone, as cortical thickness or thickening, distance, spatial arrangement and structure of trabecular organization, also providing a measure of the muscle mass and therefore an indication of the muscle/bone ratio (164).
The bone ultrasound (QUS), with measurement of parameters such as the speed of sound (SOS) and the broadband attenuation (BUA) of ultrasounds beam through the bone, is able to provide information about the elasticity and bone microarchitecture that contribute to the occurrence of fragility fracture (165).
Since the condition of cancer therapy-induced hypogonadism increases bone resorption, promoting bone loss and thereby increasing the risk of fractures, we thought to be appropriate to introduce into our database both DXA, needed to properly assess the bone quantity, and pQCT and QUS methods, useful for the qualitative assessment of bone mass, as described at Table 3.

4th field: Therapy

Aromatase is encoded by the *CYP19* gene located on chromosome 15q21.1. A tissue-specific expression of different isoforms is due to the use of different promoters and alternative splicing. Inactivating mutations of *CYP19* are associated in both sexes with an increased bone turnover and a reduced BMD. In fact, several polymorphisms of *CYP19* are known to be involved in the regulation of the aromatase activity through the stabilization of mRNA, the increase transcription or post-translational regulation of its expression (166-168). These polymorphisms include the C>T variant at the 3 'untranslated region', represented by a different repeat of a tetranucleotide sequence (TTTA)n at intron 4. Currently, data on the effect on bone of this polymorphism are still scarce, even if a new era of pharmacogenetics is an interesting perspective to identify potential subjects suitable to receive individual treatments. A study on postmenopausal Italian women showed that the allele (TTTA)12 is the most common in non-osteoporotic women, suggesting a possible protective action (169). Moreover, women with a number of repeats >11 show a higher lumbar BMD than women with a low number of repetitions, (TTTA)8-11. These data are also confirmed by studies on male individuals (170). Furthermore, in *in vitro* studies, the fibroblasts phenotype of subjects with a high number of TTTA repeats show a higher aromatase activity than cells of subjects with the opposite genotype (170).
Therefore, in this field we will: 1) collect the information required to assess the effectiveness of bisphosphonates therapy, particularly zoledronic acid in BC patients treated with anti-hormonal therapy; 2) study the BMD changes induced by these drugs, bone turnover markers, the appearance of any related fractures, and a potentially different response to treatment in relation to the presence of *ERα* and *CYP19* gene polymorphisms (Table 4).

Table 2 - 2nd Field: Oncology.

ONCOLOGY:
- Data of intervention and type of intervention
- Pathology and grading of the BC
- ER+/- and PgR+/- status
- Medical treatment of BC

Table 3 - 3rd field: Diagnosis of bone loss.

DIAGNOSIS OF BONE LOSS:
- Lumbar and femoral DXA before tamoxifen and/or AI
- Lumbar and femoral DXA after tamoxifen and/or AI
- pQCT before tamoxifen and/or AI
- pQCT after tamoxifen or AI
- QUS before tamoxifen and/or AI
- QUS after tamoxifen and/or AI
- **Bone turnover evaluation:** serum calcium and phosphate, 25OH Vitamin D, bone alkaline phosphatase, creatinine clearance, urinary calcium and phosphate excretion, deoxypiridinoline. Before tamoxifen and/or AI
- **Bone turnover evaluation:** serum calcium and phosphate, 25OH Vitamin D, bone alkaline phosphatase, creatinine clearance, urinary calcium and phosphate excretion, deoxypiridinoline. After tamoxifen and/or AI
- Fractures before tamoxifen and/or AI
- Fractures after tamoxifen and/or AI

Conclusions

The therapy with AIs in women with BC is correlated with increased loss of bone mass and fracture risk when compared to those treated with tamoxifen or placebo. The real impact of this loss on bone health will depend on the early identification of patients at risk of fracture and the application of appropriate prevention strategies. The control and measurement of the parameters needed to diagnose osteoporosis, such as family history of fractures, previous personal fractures, low BMD, physical activity, smoking habits, daily calcium and vitamin D intake, are not usually evaluated in randomized clinical trials appearing in the international literature. The-

Table 4 - 4th field: Therapy.

THERAPY:
- Lumbar and femoral DXA before anti-resorptive therapy.
- Lumbar and femoral DXA after anti-resorptive therapy
- Bone turnover evaluation: serum calcium and phosphate, 25OH Vitamin D, bone alkaline phosphatase, creatinine clearance, urinary calcium and phosphate excretion, deoxypiridinoline. Before anti-resorptive therapy
- Bone turnover evaluation: serum calcium and phosphate, 25OH Vitamin D, bone alkaline phosphatase, creatinine clearance, urinary calcium and phosphate excretion, deoxypiridinoline. After anti-resorptive therapy
- Fractures before anti-resorptive therapy
- Fractures after anti-resorptive therapy

refore, we have decided to introduce such parameters into our database. However, further studies will be necessary to document which is the most appropriate therapy for these patients. Such studies will also require more extended follow-up periods to evaluate the efficacy and toxicity of bisphosphonates before considering them as first line treatment in BC patients. Moreover, the increasing knowledge on major genes and genetic pathways involved in the pathogenesis of osteoporosis or altering the response to therapy, will be helpful to prepare preventive strategies and appropriate treatment on the basis of the pharmacogenetics findings. A greater knowledge, due to the collection of a large amount of information obtainable from an appropriate and dedicated database, may help to identify risk factors for bone loss and the adequate therapeutic choice, providing the opportunity to build a feasible, effective and homogeneous diagnostic-therapeutic path, providing also the opportunity for a preventive action to the development of osteopenia/osteoporosis in patients with BC.

Acknowledgments

This review was supported by an unrestricted grant from F. I. R. M. O. Fondazione Raffaella Becagli (to MLB). No conflict of interest has to be declared.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58:71-96.
2. American Cancer Society. Cancer Facts & Figures 2008.
3. Rosen PP, Groshen S, Saigo PE, Kinne DW, Hellman S. Pathological prognostic factors in stage I (T1N0M0) and stage II (T1N1M0) breast carcinoma: a study of 644 patients with median follow-up of 18 years. J Clin Oncol. 1989; 7(9):1239-51.
4. American Cancer Society. Breast cancer facts & figures 2005-2006. Atlanta (GA): American Cancer Society Inc.; 2005.
5. Quiet CA, Ferguson DJ, Weichselbaum RR, Hellman S. Natural history of node-negative breast cancer: a study of 826 patients with long-term follow-up. J Clin Oncol. 1995; 13(5):1144-51.
6. Rosen PP, Groshen S, Kinne DW. Prognosis in T2N0M0 stage I breast carcinoma: a 20-year follow-up study. J Clin Oncol. 1991; 9(9):1650-61.
7. Satariano WA, Ragland DR. The effect of comorbidity on 3-year survival of women with primary breast cancer. Ann Intern Med. 1994; 120(2):104-10.
8. Rozenberg S, Antoine C, Carly B, Pastijn A, Liebens F. Improving quality of life after breast cancer: prevention of other diseases. Menopause Int 2007; 13(2):71-4.
9. Freedman AN, Graubard BI, Rao SR, McCaskill-Stevens W, Ballard-Barbash R, Gail MH. Estimates of the number of US women who could benefit from tamoxifen for breast cancer chemoprevention. J Natl Cancer Inst. 2003; 95(7):526-32.
10. Da cunha EFF, Martins RCA, Albuquerque MG, de Alecastro RB. LIV-3D-QSAR Model for Estrogen Receptor Ligands. J. Mol.model. 2004; 10 297-304.
11. McDonnell DP, Norris JD. Connections and regulation of the human estrogen receptor. Science. 2002; 296(5573):1642-4.
12. McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: Cellular and molecular biology. Endocr Rev. 1999; 20(3):321-44.
13. Kushner PJ, Agard DA, Greene GL, Scanlan T S, Shiau AK, Uht RM, Webb P. Estrogen receptor pathways to AP-1. J Steroid Biochem Mol Biol. 2000; 74(5):311-7.
14. Watanabe T, Inoue S, Hiroi H, Orimo A, Kawashima H, Muramatsu M. Isolation of estrogen-responsive genes with a CpG island library. Mol Cell Biol. 1998; 18(1):442-9.
15. Dubik D, Shiu RP. Mechanism of estrogen activation of c-myc oncogene expression. Oncogene. 1992; 7(8):1587-94.
16. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell. 2000; 103(6):843-52.
17. Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. Science. 2002; 295(5564):2465-8.
18. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem. 1994; 63:451-86.
19. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 1997; 138(3):863-70.
20. Shang Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. Nat Rev Cancer. 2006; 6(5):360-8.
21. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science. 1995; 270(5241):1491-4.
22. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P. Functional domains of the human estrogen receptor. Cell. 1987; 51(6):941-51.
23. Moras D, Gronemeyer H. The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol. 1998; 10(3):384-91.
24. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature. 1997; 389(6652):753-8.
25. Gradishar WJ, Jordan VC. Clinical potential of new antiestrogens. J Clin Oncol. 1997; 15(2):840-52.
26. Harris HA, Albert MN, Leathurby Y, Malamas MS, Mewshaw RE, Miller CP, Kharode JP, Marzolf J, Komm BS, Winneker RC, Frail DE, Henderson RA, Zhu Y, Keith JC Jr. Evaluation of an estrogen receptor-ß agonist in animal models of human disease. Endocrinology. 2003; 144(10):4241-9.
27. Beato M, Sanchez-Pacheco A. Interaction of steroid hormone receptors with the transcription initiation complex. Endocr Rev. 1996; 17(6):587-609.
28. Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS, Tung L. Nuclear receptor coactivators and corepressors. Mol Endocrinol. 1996; 10(10):1167-77.
29. Glass CK, Rose DW, Rosenfeld MG. Nuclear receptor coactivators. Curr Opin Cell Biol. 1997; 9(2):222-32.
30. Feng W, Ribeiro RC, Wagner RL, Nguyen H, Apriletti JW, Fletterick RJ, Baxter JD, Kushner PJ, West BL. Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. Science. 1998; 280(5370):1747-9.
31. Heery DM, Kalkhoven E, Hoare S, Parker MG. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature. 1997; 387(6634):733-6.
32. Russo IH, Russo J. Role of hormones in cancer initiation and progression. J Mammary Gland Biol Neoplasia. 1998; 3(1):49-61.
33. Huang A, Kaley G. Gender-specific regulation of cardiovascular function: estrogen as key player. Microcirculation. 2004; 11(1):9-38.

34. Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev. 2002; 23(3):279-302.
35. Maggi A, Ciana P, Belcredito S, Vegeto E. Estrogens in the nervous system mechanisms and nonreproductive functions. Annu Rev Physiol. 2004; 66:291-313.
36. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288(3):321-33.
37. Gehrig PA, Bae-Jump VL, Boggess JF, Groben PA, Fowler WC Jr, Van Le L. Association between uterine serous carcinoma and breast cancer. Gynecol Oncol. 2004; 94(1):208-11.
38. Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, Rodabough RJ, Gilligan MA, Cyr MG, Thomson CA, Khandekar J, Petrovitch H, McTiernan A. WHI Investigators. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausual women: the Women's Health Initiative randomized trial. JAMA. 2003; 289(24):3243-53.
39. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. Cancer Res. 1988; 48(2):246-53.
40. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. Lancet 1896; 2:104-7.
41. Key TJ, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol. 1988; 24(1):29-43.
42. Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, Kahle L, Campbell WS, Tangrea JA, Schatzkin A. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev. 1996; 5(7):533-9.
43. Russo J, Hasan Lareef M, Balogh G, Guo S, Russo IH. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. J Steroid Biochem Mol Biol. 2003; 87(1):1-25.
44. Yue W, Santen RJ, Wang JP, Li Y, Verderame MF, Bocchinfuso WP, Korach KS, Devanesan P, Todorovic R, Rogan E G, Cavalieri EL. Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. J Steroid Biochem Mol Biol. 2003; 86(3-5):477-86.
45. Riggs BL, Hartmann LC. Selective estrogen-receptor modulators mechanisms of action and application to clinical practice. N Engl J Med. 2003; 348(7):618-29.
46. Katzenellenbogen BS, Montano MM, Ediger TR, Sun J, Ekena K, Lazennec G, Martini PG, McInerney EM, Delage-Mourroux R, Weis K, Katzenellenbogen JA. Estrogen receptors: Selective ligands, partners, and distinctive pharmacology. Recent Prog Horm Res. 2000; 55:163-93; discussion 194-5.
47. McKenna NJ, O'Malley BW. An issue of tissues: divining the split personalities of selective estrogen receptor modulators. Nat Med. 2000; 6(9):960-2.
48. Dowers TS, Qin ZH, Thatcher GR, Bolton JL. Bioactivation of Selective Estrogen Receptor Modulator (SERMs). Chem Res Toxicol. 2006; 19(9):1125-37.
49. Sharma M, Shubert DE, Lewis J, McGarrigle BP, Bofinger DP, Olson JR. Biotransformation of tamoxifen in a human endometrial explant culture model. Chem Biol Interact. 2003; 146(3):237-49.
50. Fisher B, Redmond C. New perspective on cancer of the contralateral breast: A marker for assessing tamoxifen as a preventive agent 1991. J Natl Cancer Inst. 1991; 83(18):1278-80.
51. Cole MP, Jones CT, Todd ID. A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. Br J Cancer. 1971; 25(2):270-5.
52. Fisher B, Costantino J, Redmond C, Poisson R, Bowman D, Couture J, et al. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. N Engl J Med 1989; 320(8):479-84.
53. Fisher B, Dignam J, Wolmark N, Wickerham DL, Fisher ER, Mamounas E, Smith R, Begovic M, Dimitrov NV, Margolese RG, Kardinal CG, Kavanah MT, Fehrenbacher L, Oishi RH. Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. Lancet 1999; 353(9169):1993-2000.
54. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; 90(18):1371-88.
55. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst. 1994; 86(7):527-37.
56. Jordan VC. Tamoxifen: Toxicities and drug resistance during the treatment and prevention of breast cancer. Annu Rev Pharmacol Toxicol. 1995; 35:195-211.
57. King CM. Tamoxifen and the induction of cancer. Carcinogenesis. 1995; 16(7):1449-54.
58. Vancutsem PM, Lazarus P, Williams GM. Frequent and specific mutations of the rat p53 gene in hepatocarcinomas induced by tamoxifen. Cancer Res. 1994; 54(14):3864-7.
59. Fendl KC, Zimniski SJ. Role of tamoxifen in the induction of hormone-independent rat mammary tumors. Cancer Res. 1992; 52(1):235-7.
60. Roos W, Oeze L, Loser R, Eppenberger U. Antiestrogenic action of 3-hydroxytamoxifen in the human breast cancer cell line MCF-7. J Natl Cancer Inst. 1983; 71(1):55-9.
61. White IN, de Matteis F, Davies A, Smith LL, Crofton-Sleigh C, Venitt S, Hewer A, Phillips DH. Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57BL/6 mice and in human MCL-5 cells. Carcinogenesis. 1992; 13(12):2197-203.
62. Pace P, Jarman M, Phillips D, Hewer A, Bliss J, Coombes RC. Idoxifene is equipotent to tamoxifen in inhibiting mammary carcinogenesis but forms lower levels of hepatic DNA adducts. Br J Cancer. 1997; 76(6):700-4.
63. Kim SY, Suzuki N, Laxmi YR, Shibutani S. Genotoxic mechanism of tamoxifen in developing endometrial cancer. Drug Metab Rev. 2004; 36(2):199-218.
64. Johnston SR. Endocrine manipulation in advanced breast cancer: Recent advances with SERM therapies. Clin Cancer Res. 2001; 7(12 Suppl):4376s-4387s; discussion 4411s-4412s.
65. Phillips C. STAR results: Raloxifene as effective as tamoxifen, better safety profile. NCI Cancer 2006; Bulletin 3 1-2.
66. Gradishar W, Glusman J, Lu Y, Vogel C, Cohen FJ, Sledge GW Jr. Effects of high dose raloxifene in selected patients with advanced breast carcinoma. Cancer. 2000;88(9):2047-53.
67. McMeekin DS, Gordon A, Fowler J, Melemed A, Buller R, Burke T, Bloss J, Sabbatini P. A phase II trial of arzoxifene, a selective estrogen response modulator, in patients with recurrent or advanced endometrial cancer. Gynecol Oncol. 2003; 90(1):64-9.
68. Chan S. Arzoxifene in breast cancer. Eur J Cancer. 2002; 38 Suppl 6:S55-6.
69. Thomas W, Burke MD, Walker CL. Arzoxifene as therapy for endometrial cancer. Gynecol Oncol. 2003; 90(2 Pt 2):S40-6.
70. Suh N, Glasebrook AL, Palkowitz AD, Bryant HU, Burris LL, Starling JJ, Pearce HL, Williams C, Peer C, Wang Y, Sporn MB. Arzoxifene, a new selective estrogen receptor modulator for chemoprevention of experimental breast cancer. Cancer Res. 2001; 61(23):8412-5.
71. Soule HD, Vazguez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. Natl Cancer Inst. 1973;51(5):1409-16.
72. Gauthier S, Caron B, Cloutier J, Dory YL, Favre A, Larouche D, Mailhot J, Ouellet C, Schwerdtfeger A, Leblanc G, Martel C, Simard J, Merand Y, Belanger A, Labrie C, Labrie F. (S)-(+)-4-[7-(2,2-dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl) ethoxy] phenyl]-2H-1-benzopyran-3-yl]phenyl 2,2-dimethylpropanoate (EM-800): A highly potent, specific, and orally active nonsteroidal antiestrogen. J Med Chem. 1997; 40(14):2117-22.
73. Simard J, Labrie C, Belanger A, Gauthier S, Singh SM, Merand Y, Labrie F. Characterization of the effects of the novel non-steroidal an-

tiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells in vitro. Int J Cancer. 1997; 73(1):104-12.
74. Simard J, Sanchez R, Poirier D, Gauthier S, Singh SM, Merand Y, Belanger A, Labrie C, Labrie F. Blockade of the stimulatory effect of estrogens, OH-tamoxifen, OH-toremifene, droloxifene, and raloxifene on alkaline phosphatase activity by the antiestrogen EM-800 in human endometrial adenocarcinoma Ishikawa cells. Cancer Res. 1997; 57(16):3494-7.
75. Bruno RD, Njar VC. Targeting cytochrome P450 enzymes: a new approach in anti-cancer drug development. Bioorg Med Chem. 2007; 15(15):5047-60.
76. Mastorakos G, Valsamakis G, Paltoglou G, Creatsas G. Management of obesity in menopause: Diet, exercise, pharmacotherapy and bariatric surgery. Maturitas. 2009 Dec 29.
77. Smith IE, Dowsett M. Aromatase Inhibitors in Breast Cancer. N Engl J Med. 2003; 348(24):2431-42.
78. Goss PE, Strasser K. Aromatase Inhibitors in the Treatment and Prevention of Breast Cancer. J Clin Oncol. 2001; 19(3):881-94.
79. Geisler J, Lien EA, Ekse D, Lonning PE. Influence of aminoglutethimide on plasma levels of estrone sulphate and dehydroepiandrostenedione sulphate in postmenopausal breast cancer patients. J Steroid Biochem Mol Biol. 1997; 63(1-3):53-8.
80. Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, Sahmoud T; ATAC Trialists' Group. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet. 2002; 359(9324):2131-9.
81. Turner RT, Wakley GK, Hannon KS, Bell NH. Tamoxifen prevents the skeletal effects of ovarian hormone deficiency in rats. J Bone Miner Res 1987; 2(5):449-56.
82. Arnett TR, Lindsay R, Kilb JM, Moonga BS, Spowage M, Dempster DW. Selective toxic effects of tamoxifen on osteoclasts: comparison with the effects of oestrogen. J Endocrinol 1996; 149(3): 503-8.
83. Wright CD, Garrahan NJ, Stanton M, Gazet JC, Mansell RE, Compston JE. Effect of long-term tamoxifen therapy on cancellous bone remodeling and structure in women with breast cancer. J Bone Miner Res 1994; 9(2):153-9.
84. Powles TJ, Hickish T, Kanis JA, Tidy A, Ashley S. Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. J Clin Oncol 1996; 14(1):78-84.
85. Vehmanen L, Elomaa I, Blomqvist C, Saarto T. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 2006; 24(4):675-80
86. Eng-Wong J, Reynolds JC, Venzon D, Liewehr D, Gantz S, Danforth D, Liu ET, Chow C, Zujewski J. Effect of raloxifene on bone mineral density in premenopausal women at increased risk of breast cancer. J Clin Endocrinol Metab 2006; 91(10):3941-6.
87. Kristensen B, Ejlertsen B, Dalgaard P, Larsen L, Holmegaard SN, Transbøl I, Mouridsen HT. Tamoxifen and bone metabolism in postmenopausal low-risk breast cancer patients: a randomized study. J Clin Oncol 1994; 12(5):992-7.
88. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 1992; 326(13):852-6.
89. Grey AB, Stapleton JP, Evans MC, Tatnell MA, Ames RW, Reid IR. The effect of the antiestrogen tamoxifen on bone mineral density in normal late postmenopausal women. Am J Med 1995; 99 (6):636-41.
90. Love RR, Barden HS, Mazess RB, Epstein S, Chappell RJ. Effect of tamoxifen on lumbar spine bone mineral density in postmenopausal women after 5 years. Arch Intern Med 1994; 154(22): 2585-8.
91. Kristensen B, Ejlertsen B, Mouridsen HT, Andersen KW, Lauritzen JB. Femoral fractures in postmenopausal breast cancer patients treated with adjuvant tamoxifen. Breast Cancer Res Treat 1996; 39(3):321-6.
92. Delmas PD, Bjarnason NH, Mitlak BH, Ravoux AC, Shah AS, Huster WJ, Draper M, Christiansen C. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N.Engl.J.Med. 1997; 337:1641-1647.
93. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene. - Results from a 3-year randomized clinical trial. JAMA 1999; 282:637-645.
94. Delmas PD, Genant HK, Crans GG, Stock JL, Wong M, Siris E, Adachi JD. Severity of prevalent vertebral fractures and the risk of subsequent vertebral and nonvertebral fractures: results from the MORE trial Bone 2003; 33:522-532.
95. Barrett-Connor E, Grady D, Sashegyi A, Anderson PW, Cox DA, Hoszowski K, Rautaharju P, Harper KD; MORE Investigators (Multiple Outcomes of Raloxifene Evaluation). Raloxifene and cardiovascular events in osteoporotic postmenopausal women. Four-years results from the MORE (Multiple Outcomes of Raloxifene Evaluation) randomized trial. JAMA 2002; 287: 847-857.
96. Eastell R, Hannon R. Long-term effects of aromatase inhibitors on bone. J Steroid Biochem Mol Biol. 2005; 95(1-5):151-4.
97. Ettinger B, Pressman A, Sklarin P, Bauer DC, Cauley JA, Cummings SR. Associations between low levels of serum estradiol, bone density, and fractures among elderly women: the study of osteoporotic fractures. J Clin Endocrinol Metab. 1998; 83(7):2239-43.
98. Buzdar AU, Robertson JF, Eiermann W, Nabholtz JM. An overview of the pharmacology and pharmacokinetics of the newer generation aromatase inhibitors anastrozole, letrozole, and exemestane. Cancer. 2002; 95(9):2006-16.
99. Mincey BA, Duh MS, Thomas SK, Moyneur E, Marynchencko M, Boyce SP, Mallett D, Perez EA. Risk of cancer treatment-associated bone loss and fractures among women with breast cancer receiving aromatase inhibitors. Clin Breast Cancer. 2006; 7(2):127-32.
100. Carney JF, Davis J. Emerging bone health issues in women with breast cancer in Hawai'i. Hawaii Med J. 2007; 66(6):164-6.
101. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hoctin-Boes G, Houghton J, Locker GY, Tobias JS; ATAC Trialists' Group. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet. 2005; 365(9453):60-2.
102. Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group, Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. Lancet Oncol. 2008; 9(1):45-53.
103. Jakesz R, Jonat W, Gnant M, Mittlboeck M, Greil R, Tausch C, Hilfrich J, Kwasny W, Menzel C, Samonigg H, Seifert M, Gademann G, Kaufmann M, Wolfgang J; ABCSG and the GABG. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: Combined results of ABCSG trial 8 and ARNO 95 trial. Lancet. 2005; 366(9484):455-62.
104. Eastell R, Hannon RA, Cuzick J, Dowsett M, Clack G, Adams JE. ATAC Trialists' group. Effect of an aromatase inhibitor on BMD and bone turnover markers: 2-year results of the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) trial. J Bone Miner Res. 2006; 21(8):1215-23.
105. Coleman RE. Group AT: Effect of anastrozole on bone mineral density: 5-year results from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. J Clin Oncol 24:5s, 2006 (suppl; abstr 511)
106. Kaufmann M, Jonat W, Hilfrich J, Eidtmann H, Gademann G, Zuna I, von Minckwitz G. Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: The ARNO 95 study. J Clin Oncol. 2007; 25(19):2664-70.
107. Boccardo F, Rubagotti A, Puntoni M, Guglielmini P, Amoroso D, Fini A, Paladini G, Mesiti M, Romeo D, Rinaldini M, Scali S, Porpiglia M, Benedetto C, Restuccia N, Buzzi F, Franchi R, Massidda B, Distante V, Amadori D, Sismondi P. Switching to anastrozole versus continued tamoxifene treatment of early breast cancer: Preliminary results of the Italian Tamoxifen Anastrozole Trial. J Clin Oncol. 2005; 23(22):5138-47.
108. Saad F, Adachi JD, Brown JP, Canning LA, Gelmon KA, Josse RG,

Pritchard KI. Cancer treatment-induced bone loss in breast and prostate cancer. J Clin Oncol. 2008; 26(33):5465-76.
109. Coates AS, Keshaviah A, Thürlimann B, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Colleoni M, Láng I, Del Mastro L, Smith I, Chirgwin J, Nogaret JM, Pienkowski T, Wardley A, Jakobsen EH, Price KN, Goldhirsch A. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: Update of study BIG 1-98. J Clin Oncol. 2007; 25(5):486-92.
110. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Cameron DA, Palmer MJ, Pater JL. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: Updated findings from NCIC CTG MA.17. J Natl Cancer Inst. 2005; 97(17):1262-71.
111. Perez EA, Josse RG, Pritchard KI, Ingle JN, Martino S, Findlay BP, Shenkier TN, Tozer RG, Palmer MJ, Shepherd LE, Liu S, Tu D, Goss PE. Effect of letrozole versus placebo on bone mineral density in women with primary breast cancer completing 5 or more years of adjuvant tamoxifen: A companion study to NCIC CTG MA.17. J Clin Oncol. 2006; 24(22):3629-35.
112. Goss PE, Qi S, Josse RG, Pritzker KP, Mendes M, Hu H, Waldman SD, Grynpas MD. The steroidal aromatase inhibitor exemestane prevents bone loss in ovariectomized rats. Bone. 2004; 34(3):384-92.
113. Goss PE, Qi S, Cheung AM, Hu H, Mendes M, Pritzker KP. Effects of the steroidal aromatase inhibitor exemestane and the nonsteroidal aromatase inhibitor letrozole on bone and lipid metabolism in ovariectomized rats. Clin Cancer Res. 2004; 10(17):5717-23.
114. Coombes RC, Kilburn LS, Snowdon CF, Paridaens R, Coleman RE, Jones SE, Jassem J, Van de Velde CJ, Delozier T, Alvarez I, Del Mastro L, Ortmann O, Diedrich K, Coates AS, Bajetta E, Holmberg SB, Dodwell D, Mickiewicz E, Andersen J, Lønning PE, Cocconi G, Forbes J, Castiglione M, Stuart N, Stewart A, Fallowfield LJ, Bertelli G, Hall E, Bogle RG, Carpentieri M, Colajori E, Subar M, Ireland E, Bliss JM; Intergroup Exemestane Study. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): A randomised controlled trial. Lancet. 2007; 369(9561):559-70.
115. Coleman RE, Banks LM, Girgis SI, Kilburn LS, Vrdoljak E, Fox J, Cawthorn SJ, Patel A, Snowdon CF, Hall E, Bliss JM, Coombes RC. Intergroup Exemestane Study group. Skeletal effects of exemestane on bone-mineral density, bone biomarkers, and fracture incidence in postmenopausal women with early breast cancer participating in the Intergroup Exemestane Study (IES): A randomised controlled study. Lancet Oncol. 2007; 8(2):119-27.
116. Asmar L, Negron A, Stokoe C, et al. The effect of tamoxifen or exemestane on bone mineral density after 2 years of adjuvant treatment of postmenopausal women with early breast cancer. 29th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 14-17, 2006.
117. Gonnelli S, Cadirni A, Caffarelli C, Petrioli R, Montagnani A, Franci MB, Lucani B, Francini G, Nuti R. Changes in bone turnover and in bone mass in women with breast cancer switched from tamoxifen to exemestane. Bone. 2007; 40(1):205-10.
118. Geisler J, Lønning PE, Krag LE, Løkkevik E, Risberg T, Hagen AI, Schlichting E, Lien EA, Ofjord ES, Eide GE, Polli A, di Salle E, Paolini J. Changes in bone and lipid metabolism in postmenopausal women with early breast cancer after terminating 2-year treatment with exemestane: A randomised, placebo-controlled study. Eur J Cancer. 2006; 42(17):2968-75.
119. Compston JE. Sex steroids and bone. Physiol Rev 2001; 81:419-47.
120. Zapantis G, Santoro N. The menopausal transition: characteristics and management. Best Pract Res Clin Endocrinol Metab 2003; 17:33-52.
121. Goss PE, Hadji P, Subar M, Abreu P, Thomsen T, Banke-Bochita J. Effects of steroidal and nonsteroidal aromatase inhibitors on markers of bone turnover in healthy postmenopausal women. Breast Cancer Res. 2007; 9(4):R52.
122. Hadji P, Ziller M, Kieback DG, Menschik T, Kalder M, Kuck J, Hasenburg A. The effect of exemestane or tamoxifen on markers of bone turnover: results of a German sub-study of the Tamoxifen Exemestane Adjuvant Multicentre (TEAM) trial. Breast. 2009; 18(3):159-64.
123. Chang J, Powles TJ, Ashley SE, Gregory RK, Tidy VA, Treleaven JG, Singh R. The effect of tamoxifen and hormone replacement therapy on serum cholesterol, bone mineral density and coagulation factors in healthy postmenopausal women participating in a randomised, controlled tamoxifen prevention study. Ann Oncol 1996; 7(7):671-675.
124. Seeman E. Pathogenesis of bone fragility in women and men. Lancet 2002; 359:1841-1850.
125. Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, Bevers TB, Kavanah MT, Atkins JN, Margolese RG, Runowicz CD, James JM, Ford LG, Wolmark N. Tamoxifen for the prevention of breast cancer: current status of theSurgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst 2005; 97(22):1652-1662.
126. Fiona M. McCaig, Lorna Renshaw, Linda Williams, Oliver Young, Juliette Murray, Elizabeth J. Macaskill, Mary McHugh, Rosemary Hannon, J. Michael Dixon. A study of the effects of the aromatase inhibitors anastrozole and letrozole on bone metabolism in postmenopausal women with estrogen receptor-positive breast cancer. Breast Cancer Res Treat. 2010; 119(3):643-51.
127. Coleman RE, Banks LM, Girgis SI, Kilburn LS, Vrdoljak E, Fox J, Cawthorn SJ, Patel A, Snowdon CF, Hall E, Bliss JM, Coombes RC; Intergroup Exemestane Study group. Skeletal effects of exemestane on bone-mineral density, bone biomarkers, and fracture incidence in postmenopausal women with early breast cancer participating in the Intergroup Exemestane Study: a randomized controlled study. Lancet Oncol. 2007; 8(2):119-27.
128. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. Lancet Oncol 2008; 9:45-53.
129. Reid IR, Brown JP, Burckhardt P, Horowitz Z, Richardson P, Trechsel U, Widmer A, Devogelaer JP, Kaufman JM, Jaeger P, Body JJ, Brandi ML, Broell J, Di Micco R, Genazzani AR, Felsenberg D, Happ J, Hooper MJ, Ittner J, Leb G, Mallmin H, Murray T, Ortolani S, Rubinacci A, Saaf M, Samsioe G, Verbruggen L, Meunier PJ. Intravenous zoledronic acid in postmenopausal women with low bone mineral density. NEJM 2002; 346(9):653-61.
130. Greep NC, Giuliano AE, Hansen NM, Taketani T, Wang HJ, Singer FR. The effects of adjuvant chemotherapy on bone density in postmenopausal women with early breast cancer. Am J Med. 2003;114(8):653-9.
131. Van Poznak C, Hannon R, Clack G, et al. The SABRE (Study of Anastrozole with the Bisphosphonate RisedronatE) study: 12-month analysis. Breast Cancer Res Treat 2007; 106:502.
132. Lester JE, Gutcher SA, Ellis SP, et al. Effect of monthly oral ibandronate on anastrozole-induced bone loss during adjuvant treatment for breast cancer: One-year results from the ARIBON study. J Clin Oncol 2007; 25:16s (suppl; abstr 553).
133. Greenspan SL, Bhattacharya RK, Sereika SM, Brufsky A, Vogel VG. Prevention of bone loss in survivors of breast cancer: A randomized, double-blind, placebocontrolled clinical trial. J Clin Endocrinol Metab. 2007; 92(1):131-6.
134. Hershman DL, McMahon DJ, Crew KD, Cremers S, Irani D, Cucchiara G, Brafman L, Shane E. Zoledronic acid prevents bone loss in premenopausal women undergoing adjuvant chemotherapy for early stage breast cancer. J Clin Oncol. 2008; 26(29):4739-45.
135. Delmas PD, Balena R, Confravreux E, Hardouin C, Hardy P, Bremond A. Bisphosphonate risedronate prevents bone loss in women with artificial menopause due to chemotherapy of breast cancer: A double-blind, placebocontrolled study. J Clin Oncol. 1997; 15(3):955-62.
136. Fuleihan Gel-H, Salamoun M, Mourad YA, Chehal A, Salem Z, Mahfoud Z, Shamseddine A. Pamidronate in the prevention of chemotherapy induced bone loss in premenopausal women with breast cancer: A randomized controlled trial. J Clin Endocrinol Metab. 2005; 90(6):3209-14.
137. Gnant MF, Mlineritsch B, Luschin-Ebengreuth G, Grampp S, Kaessmann H, Schmid M, Menzel C, Piswanger-Soelkner JC, Galid A, Mittlboeck M, Hausmaninger H, Jakesz R. Austrian Breast and Colorectal Cancer Study Group. Zoledronic acid prevents cancer treatment induced bone loss in premenopausal women receiving adjuvant endocrine therapy for hormone responsive breast cancer: A report from

the Austrian Breast and Colorectal Cancer Study Group. J Clin Oncol. 2007; 25(7):820-8.
138. Gnant M, Mlineritsch B, Luschin-Ebengreuth G, et al. Bone mineral density (BMD) at 5 years after diagnosis in premenopausal patients with endocrine responsive breast cancer, after 3 years of adjuvant endocrine treatment with goserelin and tamoxifen or anastrozole or both treatments in combination with zoledronic acid: New results from ABCSG-12. 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 13-16, 2007 (abstr 26).
139. Brufsky A, Harker WG, Beck JT, Carroll R, Tan-Chiu E, Seidler C, Hohneker J, Lacerna L, Petrone S, Perez EA. Zoledronic acid inhibits adjuvant letrozole-induced bone loss in postmenopausal women with early breast cancer. J Clin Oncol. 2007; 25(7):829-36.
140. Brufsky AM, Bosserman LD, Caradonna RR, Haley BB, Jones CM, Moore HC, Jin L, Warsi GM, Ericson SG, Perez EA. The effect of zoledronic acid on aromatase-inhibitor associated bone loss in postmenopausal women with early breast cancer reciving adjuvant letrozole: The Z-FAST study 36-month follow-up. Clin Breast Cancer. 2009; 9(2):77-85.
141. Bundred NJ, Campbell ID, Davidson N, DeBoer RH, Eidtmann H, Monnier A, Neven P, von Minckwitz G, Miller JC, Schenk NL, Coleman RE. Effective inhibition of aromatase inhibitor-associated bone loss by zoledronic acid in postmenopausal women with early breast cancer receiving adjuvant letrozole: ZO-FAST study results. Cancer. 2008; 112(5):1001-10.
142. De Boer R, Eidtmann H, Lluch A, et al. The ZO-FAST trial: Zoledronic acid effectively inhibits aromatase inhibitor associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: 24 month BMD results. Breast Cancer Res Treat 2007; 106:501.
143. Hines SL, Mincey B, Dentchev T, Sloan JA, Perez EA, Johnson DB, Schaefer PL, Alberts S, Liu H, Kahanic S, Mazurczak MA, Nikcevich DA, Loprinzi CL. Immediate vs. delayed zoledronic acid for prevention of bone loss in postmenopausal women with breast cancer starting letrozole after tamoxifen N03CC. Breast Cancer Res Treat. 2009; 117(3):603-9.
144. Hines SL, Sloan JA, Atherton PJ, Perez EA, Dakhil SR, Johnson DB, Reddy PS, Dalton RJ, Mattar BI, Loprinzi CL. Zoledronic acid for treatment of osteopenia and osteoporosis in women with primary breast cancer undergoing adjuvant aromatase inhibitor therapy. Breast. 2010.
145. Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Berger ML, Santora AC, Sherwood LM. Identification and Fracture Outcomes of Undiagnosed Low Bone Mineral Density in Postmenopausal Women. Results From the National Osteoporosis Risk Assessment. JAMA. 2001; 286(22):2815-22.
146. Smith DM, Nance WE, Kang KW, Christian JC, Johnston CC Jr. Genetic factors in determining bone mass. J Clin Invest. 1973; 52(11):2800-8.
147. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest. 1987; 80(3):706-10.
148. Mundy GR. Osteoporosis. Boning up on genes. Nature 1994; 367:216-7.
149. Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA. Genetic factors in bone turnover. J Clin Endocrinol Metab. 1991; 72(4):808-13.
150. Steward TL, Ralston SH. Role of genetic factors in the pathogenesis of osteoporosis. Journal of Endocrinology 2000; 166:235-45.
151. Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med. 1989; 320(9):554-8.
152. Hansen MA, Hassager C, Jensen SB, Christiansen C. Is heritability a risk factor for postmenopausal osteoporosis? J Bone Miner Res. 1992; 7(9):1037-43.
153. Christian JC, Yu PL, Slemenda CW, Johnston CC Jr. Heritability of bone mass: a longitudinal study in aging male twins. Am J Hum Genet. 1989; 44(3):429-33.
154. Harris M, Nguyen TV, Howard GM, Kelly PJ, Eisman JA. Genetic and environmental correlations between bone formation and bone mineral density: a twin study. Bone. 1998; 22(2):141-5.
155. Adami S, Bertoldo F, Brandi ML, Cepollaro C, Filipponi P, Fiore E, Frediani B, Giannini S, Gonnelli S, Isaia GC, Luisetto G, Mannarino E, Marcocci C, Masi L, Mereu C, Migliaccio S, Minisola S, Nuti R, Rini G, Rossini M, Varenna M, Ventura L, Bianchi G. Guidelines for the diagnosis, prevention and treatment of osteoporosis Reumatismo 2009; 61(4):260-84.
156. Joan L. Bottorff, Stephanie Barclay McKeown, Joanne Carey, Rebecca Haines, Chizimuzo Okoli, Kenneth C. Johnson, Julie Easley, Roberta Ferrence, Lynne Baillie, Erin Ptolemy. Young women's responses to smoking and breast cancer risk information. Health Educ Res. 2010 Jan 15.
157. Fontana L, Shew JL, Holloszy JO, Villareal DT. Low bone mass in subjects on a long-term raw vegetarian diet Arch Intern Med. 2005; 165(6):684-9.
158. Health Evidence Bulletins Wales. Osteoporosis. Cardiff: National Assembly for Wales 2001.
159. Bruyere O, Edwards J, Reginster J-Y. Fracture prevention in postmenopausal women. Clin Evid. 2005; (13):1419-34.
160. Patterson RE, Flatt SW, Saquib N, Rock CL, Caan BJ, Parker BA, Laughlin GA, Erickson K, Thomson CA, Bardwell WA, Hajek RA, Pierce JP. Medical comorbidities predict mortality in women with a history of early stage breast cancer. Breast Cancer Res Treat. 2010 Jan 14.
161. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001; 285(6):785-95.
162. WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser 1994; 843:1-129.
163. Gehlbach SH, Bigelow C, Heimisdottir M, May S, Walker M, Kirkwood JR. Recognition of vertebral fracture in a clinical setting. Osteoporos Int. 2000; 11(7):577-82.
164. Jiang Y, Zhao J, Augat P, Ouyang X, Lu Y, Majumdar S, Genant HK. Trabecular bone mineral and calculated structure of human bone specimens scanned by peripheral quantitative computed tomography: relation to biomechanical properties. J Bone Miner Res. 1998; 13(11):1783-90.
165. Hans D, Njeh CF, Genant HK, Meunier PJ. Quantitative ultrasound in bone status assessment. Rev Rhum Engl Ed. 1998; 65(7-9):489-98.
166. Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, and Jerums G. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 1989; 320: 554-558.
167. Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 1995; 59: 97-105.
168. Siegelmann-Danieli N, Buetow KH. Constitutional genetic variation at the human aromatase gene (Cyp19) and breast cancer risk. Br J Cancer. 1999; 79(3-4):456-63.
169. Masi L, Becherini L, Gennari L, Amedei A, Colli E, Falchetti A, Farci M, Silvestri S, Gonnelli S, Brandi ML. Polymorphism of the Aromatase Gene in Postmenopausal Italian Women: Distribution and Correlation with Bone Mass and Fracture Risk. J Clin Endocrinol Metab 2001; 80:3689-98.
170. Gennari L, Masi L, Merlotti D, Picariello L, Falchetti A, Tanini A, Mavilia C, Del Monte F, Gonnelli S, Lucani B, Gennari C, Brandi ML. A polymorphic CYP19 TTTA repeat influences aromatase activity and estrogen levels in elderly men: effects on bone metabolism. J Clin Endocrinol Metab 2004; 89:2803-10.