Musculoskeletal side effects of aromatase inhibitors treatment in women with breast cancer

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To my family and friends,

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ABSTRACT

The introduction of aromatase inhibitors (AI) therapy for the treatment of postmenopausal women with hormone receptor–positive breast cancer has led to a significant improvement of patient survival. However, AI–associated side effects, whose etiology is still unexplained, compromise quality of life and lead to non–compliance, representing a significant burden. In this thesis, we focus on musculoskeletal symptoms, essentially joint pain and bone mineral density loss with the associated increase in fracture risk. In a context of everyday clinical practice, we describe the evolution of these outcomes during AI therapy. A significant increase in the intensity of joint pain has been detected. Patients also experience an accelerated bone mineral density loss which is dimmed by oral bisphosphonates. Our research leads us to state that the genetic background of the individual is essential in this setting. Thus, polymorphic variants in genes involved in steroid and vitamin D metabolic pathways may contribute to the onset and/or intensity of these undesirable symptoms. Furthermore, bone is raised for the first time as a potential steroidogenic tissue. The results are promising and reveal new insights into the functioning of these musculoskeletal events.

RESUMEN

La terapia con inhibidores de aromatasa (IA) para el tratamiento del cáncer de mama positivo para receptor de estrógenos en mujeres postmenopáusicas ha producido un aumento significativo de la supervivencia. No obstante, los efectos secundarios asociados a este tratamiento, cuya etiología es todavía desconocida, comprometen la calidad de vida de las pacientes y disminuyen la adherencia al tratamiento, representando un gran inconveniente. Esta tesis se enfoca en los síntomas musculoesqueléticos, específicamente el dolor articular y la pérdida de densidad mineral ósea con el consecuente aumento del riesgo de fractura. Se describe la evolución de estos síntomas durante la terapia con IA en la práctica clínica habitual. Se ha detectado un aumento en la intensidad del dolor articular. Además las pacientes experimentan una pérdida acelerada de la densidad mineral ósea, que puede ser atenuada con bifosfonatos orales. Nuestros estudios nos permiten afirmar que el componente genético del individuo es esencial en este contexto. Así, variantes polimórficas en genes involucrados en las vías metabólicas de los estrógenos y la vitamina D pueden contribuir a la aparición y/o intensidad de estos efectos adversos. Además, el hueso se plantea por primera vez como un tejido esteroidogénico potencial. Los resultados son prometedores y revelan nuevos conocimientos acerca del funcionamiento de estas manifestaciones musculoesqueléticas.

PREFACE

The work presented in this doctoral thesis was carried out in the Inflammatory and Cardiovascular Disorders Program of Hospital del Mar Medical Research Institute (IMIM) in Barcelona, Spain, under the supervision of Dr. Natalia Garcia Giralt and Dr. Xavier Nogués Solán.

The content of this thesis provides novel insights on the causal mechanisms underlying the side effects associated to AI treatment in postmenopausal women with breast cancer. The results presented here illustrate an important role for the genetic background in the onset and/or intensity of joint pain and bone mineral density loss associated to this medication.

CONTENTS

GLOSSARY

Abbreviation	Meaning
17βHSDs	17β–hydroxysteroid dehydrogenases enzymes
3βHSD	3β–hydroxysteroid dehydrogenase
ACTH	Adrenocorticotropic hormone
AI	Aromatase inhibitors
AIA	AI–related arthralgia
AIBL	Aromatase inhibitor induced bone loss
ASCO	American Society of Clinical Oncology
ATAC	Arimidex, Tamoxifen, Alone or in Combination trial
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
BP	Bisphosphonates
BTM	Bone turnover markers
COMT	Catechol–O–methyltransferase enzyme
CV	Coefficient of variation
CYPs	Cytochrome P450s
DBP	Vitamin D binding protein
DHC	Dehydrocholesterol
DHEA	Dehydroepiandrosterone
DHEA–S	Dehydroepiandrosterone–sulphate
DHT	Dihydrotestosterone
DXA	Dual–energy X–ray absorptiometry

ECM	Extracellular matrix
ER	Estrogen receptor
FGF	Fibroblast growth factor
FN	Femoral neck
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin releasing hormone
GWAS	Genome wide association study
HER-2	Human epidermal growth factor receptor-2
HRT	Hormone replacement therapy
HSDs	Hydroxysteroid dehydrogenases
IGF	Insulin growth factor
IL	Interleukin
INF-ɣ	Interferon gamma
IOM	Institute of medicine
LBD	Ligand binding domain
LH	Luteinizing hormone
LH-RH	Luteinizing hormone-Releasing Hormone
LRP5	Low-density lipoprotein receptor-related protein 5 gene
LS	Lumbar spine
M-CSF	Macrophage colony stimulating factor
NAD	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
OPG	Osteoprotegerin
P450c17	17α-hydroxylase/17,20-Lyase
P450scc	Cholesterol side-chain cleavage enzyme
PPI	Inorganic pyrophosphate
PR	Progesterone receptor
PTH	Parathyroid hormone
RANK-L	Receptor activator of nuclear factor-κβ
RCT	Randomized control trial

SEER	Surveillance, Epidemiology, and End Results
SERDs	Selective estrogen receptor downregulators
SERMs	Selective estrogen receptor modulators
SNPs	Single nucleotide polymorphisms
SRD5	5α–reductase enzymes
StAR	Steroidogenic acute regulatory protein
SULT	Sulfotransferase enzymes
TBS	Trabecular bone score
TCL1A	T–cell leukaemia 1A gene
TGF–β	Transforming growth factor beta
TNF–α	Tumour necrosis factor alpha
VAS	Visual analogic scale
VDR	Vitamin D receptor
$VitD_2$	Vitamin D_2
$VitD_3$	Vitamin D_3

Chapter 1

INTRODUCTION

Dios no juega a los dados con el Universo

Albert Einstein

1. BREAST CANCER

The human mammary gland is mainly composed of glandular stromal tissues. The stromal tissue includes adipose and fibrous material surrounding and supporting the lobules and ducts of the glandular tissue, responsible for milk synthesis and transport to the nipple, respectively. The breast also contains lymphatic and blood vessels (**Fig. 1**).

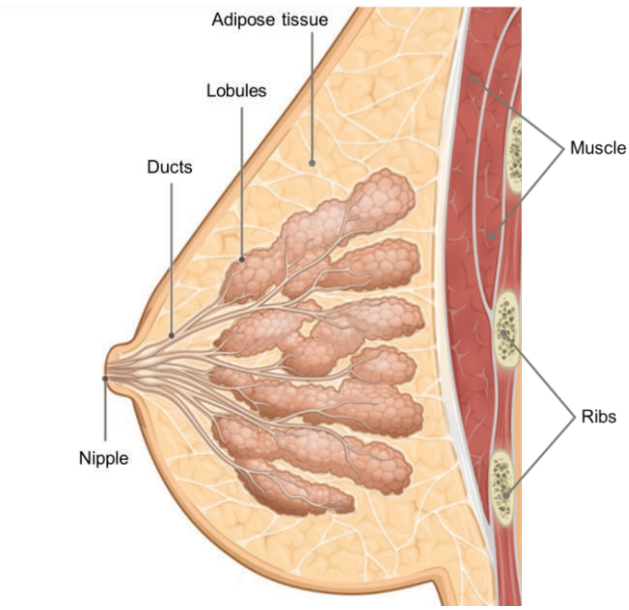

Figure 1. The anatomy of the breast. Sagittal section of the human mammary gland. Adapted from http://www.adamondemand.com/anatomy-and-physiology-of-the-breast/#.VrxpTrR97Gh

Breast cancer is a malignant tumour that starts in the cells of the breast tissue, primarily in the ducts or lobules, although a small number can originate in other tissues[1].

1.1. BREAST CANCER EPIDEMIOLOGY

Breast cancer is the most frequently diagnosed cancer in women. It is estimated that 1.7 million new cases worldwide were diagnosed in 2012, accounting for 25% of all new cancer cases in women. These data are good representative for the Spanish population (**Fig. 2**), in an intermediate situation at European level[2]. It is estimated that 1 in 8 women will develop breast cancer in their lifetime[3].

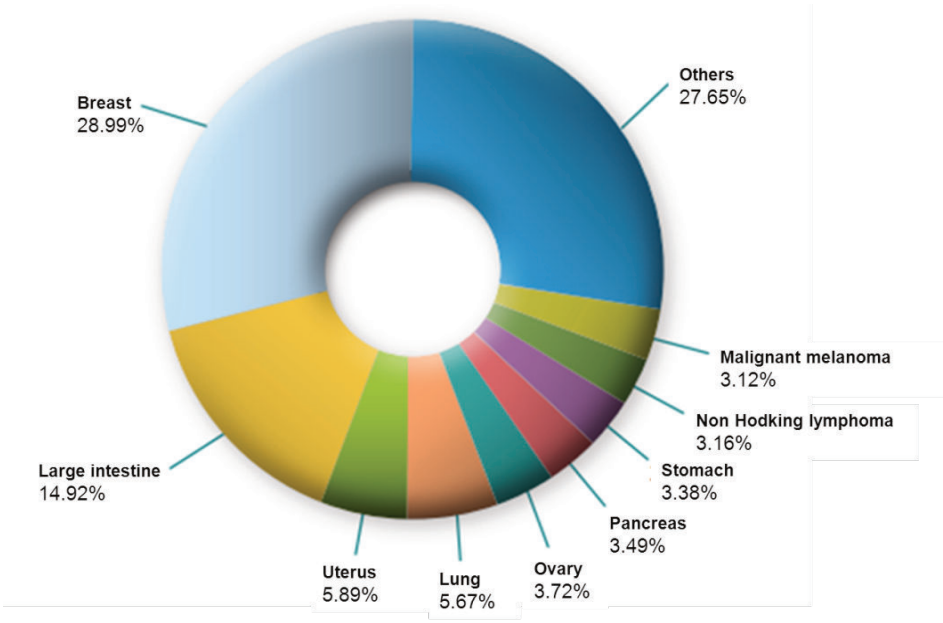

Figure 2. Estimated cancer incidence by tumour type in Spanish women for the year 2012. Adapted from http://www.seom.org

Breast cancer is the second leading cause of cancer death (following lung cancer) among women in developed countries and the first cause of cancer death among women in Spain, with 6,075 deaths in 2012[2]. Fortunately, in contrast to the increase in the incidence rate, breast cancer mortality rate in

western countries has remained stable or decreasing during the past 25 years (**Fig. 3**).

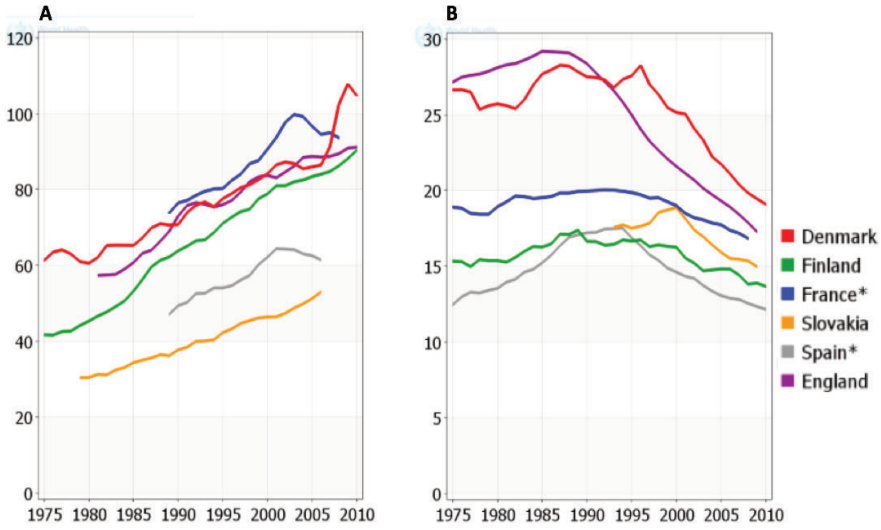

Figure 3. Trends in incidence (A) and mortality (B) from female breast cancer in European countries. Age-standardised rate per 100,000. * Regional data. Adapted from: http://globocan.iarc.fr/Default.aspx

According to the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI), the current 5-year relative survival for breast cancer patients, compared with the rest of the population, is 89%. Even those with metastatic disease have a 23% 5-year survival, on average. Earlier detection, effective screening programs and advances in therapies are among factors underlying this survival improvement[4,5].

2. BREAST CANCER CLASSIFICATION

Breast cancer variability – in terms of risk factors, clinical presentations, pathological features, response to therapy and outcomes – leads to consider that it is, in fact, a collection of different diseases affecting the same anatomical organ. Therefore, its optimal description must include several grading systems influencing prognosis and treatment response. Among them, histology[6], grade[7] and stage[8] of the tumour, proliferation rate[8] and receptor status can be highlighted.

2.1. RECEPTOR STATUS

Determination of receptor status of breast tumours is useful as a prognostic and predictive factor and has become a standard practice in the management of this neoplasm. Currently, the presence of 3 possible receptors is assessed.

2.1.1. Human epidermal growth factor receptor–2 (HER2)

HER2 is a transmembrane receptor, member of the human epidermal growth factor receptor family. Ligand binding to HER2 results in the activation of intracellular signalling pathways of enhanced cell growth, survival and differentiation. HER2 is involved in the regulation of normal breast growth and development[9]. HER2 overexpression disrupts normal control mechanisms, potentially leading to the formation of aggressive tumour cells[10] and has been associated with increased cell proliferation, cell motility, tumour invasiveness, progressive regional and distant metastases, accelerated angiogenesis and reduced apoptosis[11]. This receptor can be

valuable as an adverse prognosis indicator[12], predictive factor for response to therapy and therapeutic target[13].

2.1.2. Hormonal receptors

The biological effects of estrogens are mediated through the estrogen receptor (ER), a member of the nuclear receptor superfamily. Estrogen union to the receptors can trigger genomic and non–genomic signalling pathways, often converging in the regulation of transcriptional processes[14]. Progesterone receptors (PR) are ligand–activated transcription factors also belonging to nuclear receptor family, mediating progesterone actions.

Estrogens, progesterone, as well as other sex steroids are absolutely essential for the proliferation and morphogenesis of the mammary gland[15]. However, in the normal adult mammary gland, ER and PR are relegated to 7 – 10% of the epithelial cell population, which remain in a non–proliferative state, while normal proliferating cells are devoid of this kind of receptors[16]. By contrast, it is estimated that ER are expressed in approximately three–quarters of cases of breast tumours, reaching 80% among postmenopausal patients[17]. About 65% of breast cancers expressing ER also express PR[18]. Breast cancers expressing ER and/or PR are designated as hormone receptor–positive (or hormone sensitive) breast cancers. The ER predictive value falls on its ability to identify patients who may benefit from endocrine therapy, a pivotal treatment that works by blocking estrogen production or by interfering with hormone action[19]. Thus, with appropriate adjuvant treatment, patients with receptor–positive disease have substantially better prognoses than those with hormone receptor–negative disease[19]. For its part, PR can be useful to identify ER–/PR+ tumours, representing from 3%

to 5% of patients, as they may also respond to hormonal therapy. There are also some evidences suggesting that, PR status is an independent predictive factor for benefit from adjuvant endocrine therapy with tamoxifen[20], helping in the selection of initial adjuvant therapy[21,22]. Guidelines from American Society of Clinical Oncology (ASCO) recommend that both ER and PR analysis should be performed routinely in all invasive breast cancers, as a selection criteria for patients who should receive endocrine therapy[23]. Unfortunately, the potential of hormone–receptors as a prognostic factors declines over time, so that the beneficial effect of its presence is limited to the first 5 years after diagnosis[24,25].

3. RISK FACTORS FOR BREAST CANCER

Tremendous efforts have been made in order to understand the etiology of breast cancer. Research into the causes of the malignancy has allowed to identify several well-established risk factors and a variety of others are currently under study. These scientific inquiries agree that breast cancer is a complex, multifactorial disease where there is a strong interplay between genetic and environmental elements. Some of the most investigated risk factors are highlighted below.

3.1. GENETIC RISK FACTORS

It is estimated that the heritable factors account for 27% of all breast cancer cases[26]. Two classes of susceptibility genes exist.

3.1.1. High penetrance genes

Allelic variants conferring high individual risk of breast cancer belong to this category. The tumour suppressor genes *BRCA1*, *BRCA2*, *ATM* and *p53* are the most common high-penetrance genes[27]. Nonetheless, despite of being involved in around half of the familiar clustering of early onset breast cancer[27] they only explain 5%-10% of all breast cancers[28].

3.1.2. Low penetrance genes

In contrast to the high penetrance genes, low penetrance genes contain disease-associated variant alleles that only confer a small to moderate risk to individuals. Notwithstanding, low penetrance genes are more prevalent in the population and therefore explain a greater proportion of breast cancers

than the highly penetrant genes. Candidate low penetrance genes that have been evaluated to date include those encoding for enzymes involved in DNA repair[29], estrogen and carcinogen metabolism, and those participating in the detoxification of reactive oxygen species emerging in these reactions[30–33]. These tumours are likely to result from the interplay of many genetic and environmental factors and have a relatively late age at diagnosis.

3.2. HORMONAL RISK FACTORS

A wealth of epidemiological and experimental information points to the involvement of estrogens and other hormones in human breast cancer.

3.2.1. Reproductive factors

It has been shown repeatedly that a woman's risk for breast cancer is associated with her lifetime exposure to estrogen[34–36] which is, in turn, determined by several endogenous and exogenous variables. Reproductive history is among the most important determinants of breast cancer risk. Roughly, all those factors increasing the number of menstrual cycles are associated with an increased likelihood for developing breast cancer.

- Age at first live birth: increasing maternal age at first live birth is associated with increased risk for developing the disease. In women having the first child at 20, the risk of breast cancer is about half that of those having the first child at 30[37].

- Number of births: the risk of breast cancer declines with the number of children borne. Women who have undergone 5 or more full-term pregnancies have a 50% reduced risk of developing the malignancy

when compared with those who have not given birth[38].

- Duration of lactation: breastfeeding, regardless of duration or timing, has also been found to be protective against developing breast cancer[39].

- Age at menarche and menopause: early menarche and late menopause are known to increase women's risk of developing breast cancer. The risk increases by almost 3% for each year older at menopause[40]. Thus, women who have attained menopause at 55 years rather than 45 years, have approximately 30% higher risk. Breast cancer risk also increases by 5% for every year younger at menarche[31].

3.2.2. Other hormonal factors

- Body mass index (BMI): overweight and obesity have been related with an increased risk of postmenopausal breast cancer[41]. When the ovaries cease to produce estrogen, adipose tissue becomes the major source of estrogen in postmenopausal women. Although the relationship between BMI and breast cancer is not completely established, it may result, at least in part, from the increased levels of estrogens in obese women[42].

- Exogenous estrogens: the association of exogenous hormones, primarily hormonal contraceptives and hormone replacement therapy (HRT), with breast cancer has been widely studied. The difficulty to reach a consensus may be attributed in part to the variability of the exposures. Changes in patterns of use, reductions in hormone dose and temporal considerations all contribute to the difficulty to compare the many studies. Despite the conflicting results obtained, a combined analysis of 54 studies concluded that current use of oral contraceptives

poses a slight (24%) increase in the risk, which disappears 10 years after the cessation of use[43]. The use of HRT as a risk factor for developing breast cancer has also been controversial. Overall, some strong evidences suggest risk increases with long-term use[44,45].

- Androgens: Normal and malignant mammary epithelial cells also have androgen receptors (AR), indicating specific responsiveness to androgens. Besides estrogens, elevated endogenous androgens have also long been implicated as a potential risk factors for breast cancer[46].

3.3. NON-HORMONAL RISK FACTORS

A number of non-hormonal risk factors have been associated with the development of breast cancer; however, some of them may also be indirectly tied to modulation of estrogen exposure.

3.3.1. Age

One of the best-documented risk factors for breast cancer is age, which is considered to be a surrogate for DNA damage accumulated during life. The disease is less common in women younger than 30 years, after which it increases sharply until the age of 80. At that point the incidence rates flatten out and then start to decline[3].

3.3.2. Geographical variation and ethnicity

Age-adjusted incidence and mortality for breast cancer vary by up to a factor of five between countries. The highest breast cancer incidence rates are observed in high-income nations, including countries in North-America, Australia and Northern and Western Europe. Conversely, the lowest

incidence rates are detected in Middle and Eastern Africa, Eastern and South–Central Asia and Central America[47].

Data from SEER[1] program of the NCI indicate that white women have the highest rate of getting breast cancer, followed by African–American, Asian/Pacific Islander, Hispanic and American Indian/Alaska Native women. However, differences in breast cancer incidence rates between most racial/ethnic groups can be largely explained by differences in other risk factors[48]. Moreover, studies show that migrants assume the rate in the host country within one or two generations, indicating that environmental factors are of greater importance than genetic factors[49].

3.3.3. Radiation

Mammography has convincingly demonstrated to result in a clear reduction in death from breast cancer for women over the age of 50. However, some studies have demonstrated that low–dose radiation from mammographic equipment indeed increases breast cancer risk, especially in those high–risk women[50]. Overall, it seems that the risk–benefit equation is clearly in favour of such screening programs[51].

3.3.4. Previous benign disease

Breast cancer risk in women with severe atypical epithelial hyperplasia is 4 to 5 times higher than in women who do not present any benign proliferative changes[52]. In fact, mammographic density, referring to the amount of radiologically dense breast–tissue appearing on a mammogram, represents one of the most important risk factors for breast cancer[53]. However, if breast density is an independent risk factor is still under debate. Some

studies note that it may serve as a marker for other factors, such as the history of estrogen exposure[54].

3.3.5. Lifestyle and dietary factors

The International Agency for Research on Cancer estimates that 25% of breast cancer cases worldwide are due to overweight/obesity and a sedentary lifestyle[55]. Certain modifiable lifestyle behaviours can affect the risk of the disease.

- Alcohol consumption: taken as a whole, the studies suggest that alcohol consumption at a level of 1 to 2 drinks per day modestly increases breast–cancer risk[56]. The mechanism underlying the carcinogenic effect associated with alcohol is not still understood. However, some studies suggest that alcohol consumption increases the exposure to estrogen, leading to an induction of radical oxygen species production[57] and adduct formation[58].

- Dietary factors: it is well known that measurement of dietary intake is inexact and prone to misclassification. However, a large number of factors have been described to increase (fat[59], meat[60]) or reduce (fruits and vegetables[40], soy protein[61] and vitamins A, C, E and D[62]) breast cancer risk.

- Exercise: physical activity has been proposed to have a beneficial effect on breast cancer risk[63]. Apparently, physical activity could exert its influence on the disease through an estrogen–dependent mechanism, by decreasing the total number of ovulatory cycles[64].

4. ESTROGENS AND BREAST CANCER

Already over 100 years ago – when Dr. George Beatson described the regression of metastatic breast cancer after ovarian ablation[65] – the influence of hormonal factors in breast cancer was recognized. Since then, a wealth of epidemiologic and experimental information has pointed to the involvement of estrogens, and other reproductive hormones, in human breast cancer[46,66–68]. Person–to–person differences in synthesis, regulation as well as conjugation pathways of sex steroids may define subpopulations of women with higher lifetime exposure to hormone–dependent growth promotion or to cellular damage from particular estrogens and/or estrogen metabolites. Such variation could explain a portion of the cancer susceptibility associated with hormone exposure.

4.1. STEROID BIOSYNTHESIS

Steroidogenesis is the complex multi–enzyme process by which cholesterol is converted to biologically active steroid hormones. The greatest supply of cholesterol to steroidogenesis comes from plasma low–density lipoproteins derived from dietary cholesterol[69].

4.1.1. Cholesterol transport

The first step in steroidogenesis takes place within mitochondria. However, cholesterol needs to be transferred from the outer mitochondrial membrane to the inner membrane. The aqueous phase between these two membranes cannot be crossed by the lipophilic cholesterol by itself. Although the mechanisms by which cholesterol is transported within the

mitochondria are not completely understood, it is now known that the process is primarily mediated by the action of the steroidogenic acute regulatory protein (StAR).

4.1.2. Cytochrome P450 and HSDs

Biosynthesis of estrogens involves series of enzymatic steps from cholesterol to androgens and estrogens. Most enzymes involved in steroid biosynthesis are either cytochrome P450s (CYPs) or hydroxysteroid dehydrogenases (HSDs).

Cytochrome P450 is a generic term for a superfamily of oxidative enzymes containing a single heme cofactor[70]. The term P450 (pigment 450) is derived from their ability to absorb light at 450 nm in their reduced states complexed with carbon monoxide. Human CYPs are primarily membrane–associated proteins either of the inner membrane of the mitochondria (type I) or of the endoplasmic reticulum (type II). All P450 enzymes activate molecular oxygen using their heme centre and add electrons from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). P450 enzymes are associated with the oxidative metabolism of a large number and variety of organic compounds, both endogenous and exogenous[71]. These reactions are mechanistically and physiologically irreversible[72]. The human genome includes genes for 57 CYPs divided among 18 families[73]. Six CYP enzymes are involved in steroidogenesis.

HSDs are enzymes that interconvert active and relatively inactive forms of individual steroid hormones using nicotinamide cofactors NADP+/NADPH and NAD+/NADH[74]. Based on their activities, the HSDs can be classified as dehydrogenases or reductases. These enzymes tend to function in one

direction, determined by the available cofactors[74]. Unlike P450 enzymes, each of the reactions catalysed by HSDs can be conducted by at least two isoenzymes[75].

4.1.3. Steroidogenesis

The conversion of cholesterol to pregnenolone by the cholesterol side-chain cleavage enzyme (P450scc) is the initial step in the biosynthesis of sex-steroid hormones (**Fig. 4**). P450scc is one of the few CYPs localized in the mitochondria and catalyses the conversion in three monooxygenase reactions, with the involving of two other proteins for the electron transfer: ferredoxin and ferredoxin reductase. Although this reaction is considered the rate-limiting step of steroidogenesis, P450scc is always active. Indeed, its activity is limited by the true rate-limiting step of the pathway: the supply of cholesterol in the inner membrane by StAR.

Once pregnenolone has been synthetized it can leave the mitochondria to be converted to progesterone, by 3β-hydroxysteroid dehydrogenase (3βHSD) in the microsomal compartment[76]. An alternative option for pregnenolone, is to undergo the activities of 17α-hydroxylase/17,20-lyase (P450c17), thus being first hydroxylated to 17-OH-pregnenolone and then converted to dehydroepiandrosterone (DHEA).

It should be noted that progesterone can also be the substrate for P450c17[77], resulting in the production of 17-OH-progesterone[77], the precursor of glucocorticoids[78]. However, the 17,20-lyase activity of P450c17 is not efficient for the conversion 17-OH-progesterone to androstenedione[77], so that under normal circumstances, progesterone is not an important precursor for human sex steroid synthesis.

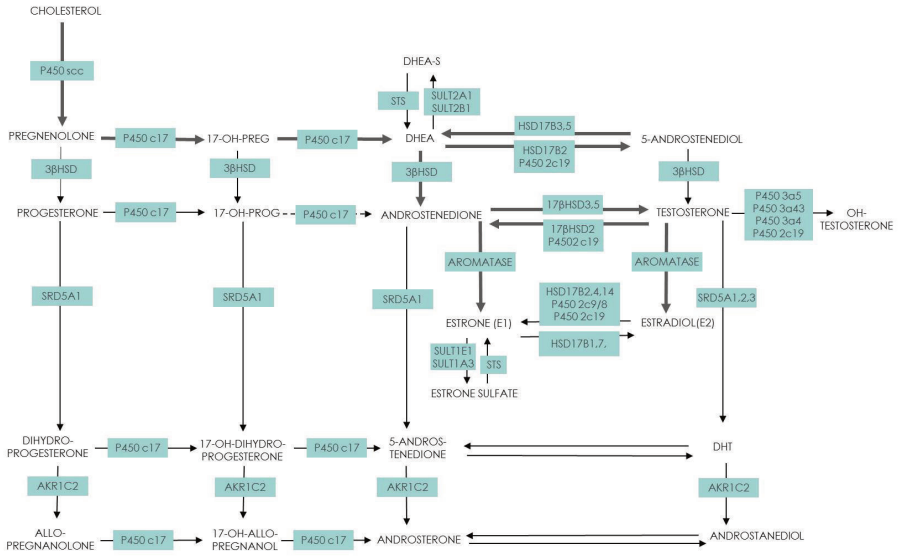

Figure 4. Steroidogenic pathway. Arrows in bold indicate the classical synthesis pathway. The existence of an alternative pathway (non-bold arrows) to the active androgen dihydrotestosterone (DHT), has been described. This "backdoor" pathway occurs in those steroidogenic tissues expressing both P450c17 and the 5α-reductase enzymes (SRD5).

3βHSD, also acts upon DHEA to generate androstenedione[76]. Both DHEA and androstenedione are substances of weak androgenic activity which serve predominantly as a precursors for more potent androgens such as testosterone[79]. The interconversion of androstenedione and testosterone, as well as multiple other reactions, are associated to the 17β-hydroxysteroid dehydrogenases enzymes (17βHSDs). The specific step from androstenedione to testosterone is carried out by 17βHSD3[80] and 17βHSD5[81], while the opposite process, that is, inactivation of testosterone, is mainly carried out by 17βHSD2[82].

Finally, estrogens are produced by the aromatization of androgens,

androstenedione and testosterone, by a complex series of reactions catalysed by P450c19, also known as aromatase (**Fig. 4**).

Multiple estrogen metabolites have been identified. However, in general, only 4 estrogen hormones are considered of interest: estradiol, estrone, estrone sulphate and estriol[83], being the latter of the exclusively importance in pregnant women. The interconversion of estradiol and estrone is also possible with the intervention of 17βHSDs enzymes. Moreover, steroid sulphates, as DHEA–sulphate (DHEA–S) and estrone–sulphate may be formed by sulfotransferase enzymes (SULT)[84]. Thus, while estrone and estrone–sulphate are inactive by itself, a substantial degree of estradiol may be synthesized by reduction of estrone[85] (**Fig. 4**). However, the relative contribution of this pathway to plasma and tissue estradiol has not been yet disentangled.

4.2. STEROIDOGENIC TISSUES AND REGULATION

A tissue is said to be steroidogenic if it is able to convert cholesterol into pregnenolone via P450scc. In women, classic steroidogenic tissues include the adrenal glands and ovaries[86].

The regulation of steroidogenesis can be divided into 3 key events in the steroidogenic pathway. Unlike other secretory tissues, steroidogenic cells store very little amounts of steroids[75]. Thus, a rapid steroidogenic response is needed. Acute regulation is mediated by StAR protein, facilitating the rapid influx of cholesterol into mitochondria. Chronic/quantitative regulation is principally at the level of transcription of P450scc, which is the enzymatically rate–limiting step. Finally, qualitative regulation, determining

the class of steroid produced, is principally determined by P450c17, the key branch point in steroid hormone synthesis, leading to tissue–specific steroid synthesis depending on the presence/absence of its activities[75].

Moreover, in each steroidogenic cell, the pattern of steroid products secreted is regulated by different hormonal tissue–specific systems. In fact, diagrams of steroidogenic pathway differ in each steroidogenic cell type[75].

Originally considered to be a major source of circulating estrogens in postmenopausal women, the adrenal cortex is the main source of circulating androgens (**Fig. 5**).

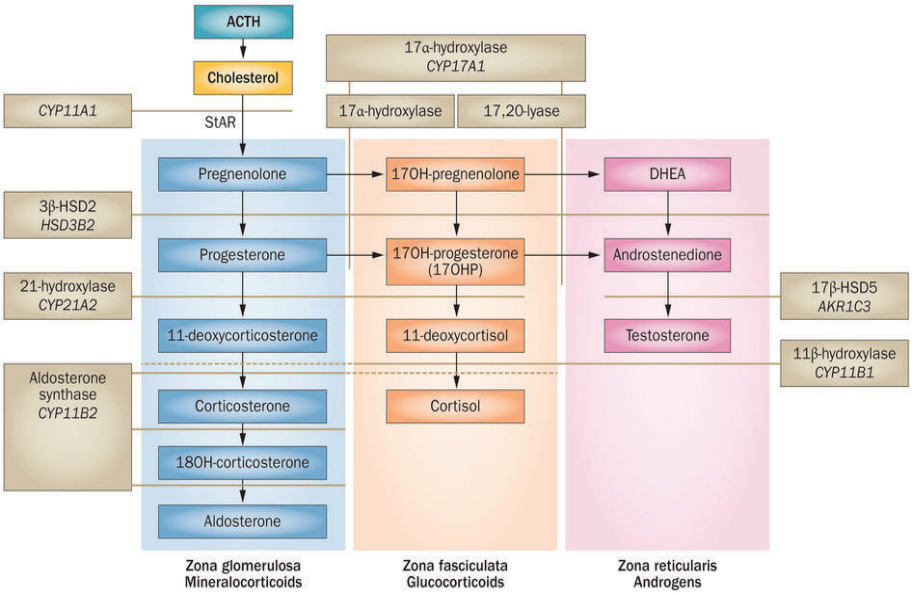

Figure 5. Steroid synthesis in adrenal glands. The presence/absence of some strategic enzymes discussed above, determine the ability of the 3 different adrenal layers to synthesize steroid products. The zona glomerulosa produces aldosterone under regulation by the renin/angiotensin system[87]. The zona fasciculata mainly produces cortisol and corticosterone under the influence of the adrenocorticotropic

hormone (ACTH)[75]. Finally, the adrenal zona reticularis express large amounts of P450scc, so that DHEA is produced, much of which is sulphated to DHEAS by SULT2A1. Moreover, small amounts of DHEA are converted to androstenedione, and very small amounts of this androstenedione are converted to testosterone[75]. Extracted from Han et al. (Nature Reviews Endocrinology, 2014)[88].

In contrast to the adrenal gland, the ovary cannot produce glucocorticoids or mineralocorticoids because it lacks the enzymes 21–hydroxylase and 11β–hydroxylase. In the ovary, the synthesis and release of estrogens are centrally regulated by the hypothalamus–pituitary–gonadal axis (**Fig. 6**).

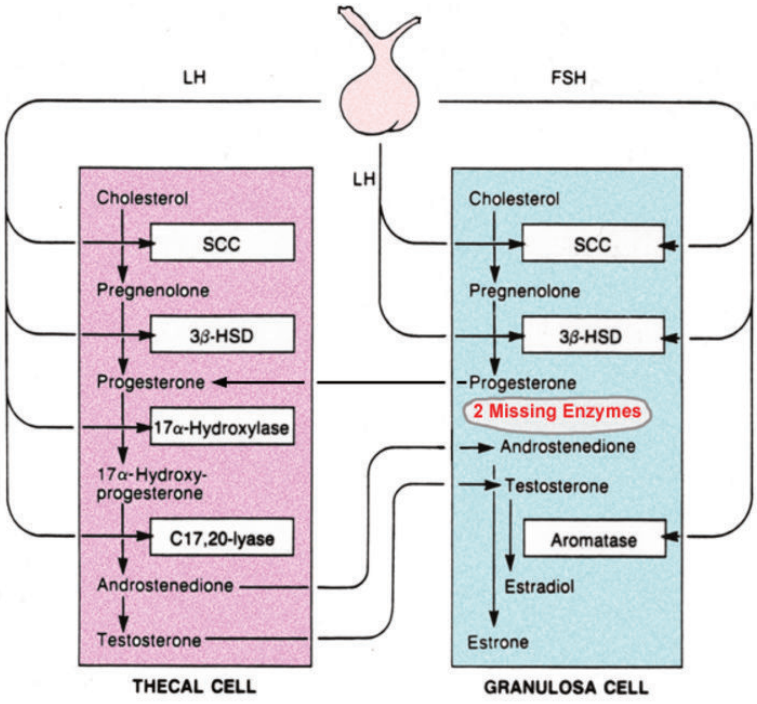

Figure 6. Steroid synthesis by cooperation of cells in the ovarian follicle. Differential regulation by luteinizing hormone (LH) and follicular stimulating hormone (FSH) of ovarian estrogen, progesterone and androgen production. In general terms, LH stimulates the expression of P450scc in granulosa cells, inducing pregnenolone and also progesterone synthesis. Granulosa cells do not express P450c17 and

consequently, pregnenolone and progesterone diffuse into adjacent theca cells. Once there, they can be converted to androstenedione by P450c17 and 3βHSD2. Small amounts of this androstenedione are secreted or converted to testosterone, but most androstenedione returns to the granulosa cells where it is converted to estrone and then to estradiol, under the influence of FSH[75]. Extracted from http://kcampbell.bio.umb.edu/MamTox/Presentations/Session3/ Session%203.html

Hypothalamus, secretes gonadotrophin releasing hormone (GnRH), which in turn acts on pituitary to release LH and FSH. These two hormones act on gonads to produce estrogen in a cyclic manner during menstrual cycle. Steroidogenesis in the ovary is also complex because the enzymatic steps are partitioned between the granulosa and theca cells (**Fig. 6**).

Although *de novo* synthesis from cholesterol is restricted to a limited number of sites, estrogens can also be synthesized from their circulating androgen precursors in a number of extragonadal tissues expressing the aromatase enzyme, such as the mesenchymal cells of adipose tissue including that of the breast, osteoblasts and chondrocytes of bone, the vascular endothelium and aortic smooth muscle cells, and numerous sites in the brain[89]. The quantitative contribution of this synthesis to circulating estrogen levels has not been elucidated.

4.3. STEROIDS IN POSTMENOPAUSAL WOMEN

Menopause is characterized by a marked reduction in the amount of circulating estrogens. Thus, when the ovaries cease functioning, aromatization of androgens by peripheral tissues becomes the responsible mechanism for estrogen synthesis. Although the total amount of estrogen synthesized by these extragonadal sites may be small, the local tissue

concentrations achieved are probably high and exert biological influence locally. Under these circumstances, estradiol is not just an endocrine factor but acts as paracrine or even intracrine factor[90], playing an important physiological role.

We have gained only a limited understanding of which factors determine plasma and tissue estrogen concentrations in postmenopausal women. Aromatase is the product of *CYP19A1*, a gene spanning 10 exons. There are at least 10 variants of exon I that can be spliced into the 5'–untranslated region in a tissue–specific fashion[91] (**Fig. 7**). Thus for example, the promoter from exon I.4 is preferably utilized for adipose tissue and bone.

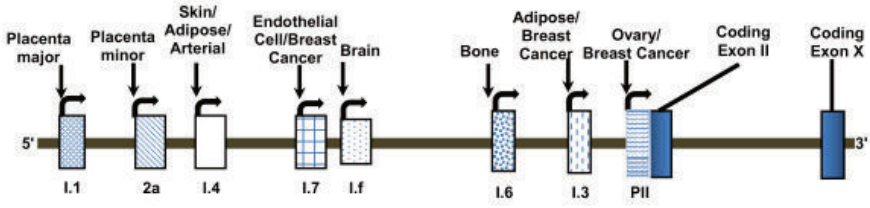

Figure 7. Partial structure of human aromatase gene. Human aromatase gene is located on chromosome 15. The aromatase gene is ~123 kb long and contains nine coding exons (II–X). Partially tissue specific promoters direct aromatase gene transcription. Extracted from: Khan et al. (Reproductive Biology and Endocrinology, 2011)[92].

Moreover, a phenomenon of promoter switching depending upon different regulatory factors, including hormones, cytokines and cell differentiation inducers has been shown in many tissues.

Testosterone and estradiol circulate in the bloodstream, bound mostly to the sex–hormone binding globulin[93], which influence their bioavailability.

Therefore, only a very small fraction of about 1–2% is unbound, or "free", and thus biologically active[94].

4.4. STEROID MECHANISMS OF BREAST CARCINOGENESIS

Several studies have demonstrated that estrogens are carcinogens in various tissues, including the kidneys, liver, uterus, and mammary glands[95,96]. Two different but complementary pathways are likely contributing to the carcinogenicity of estrogen.

4.4.1. ER signalling.

Most of the actions of estrogens are mediated via intracellular ERs (**Fig. 8**).

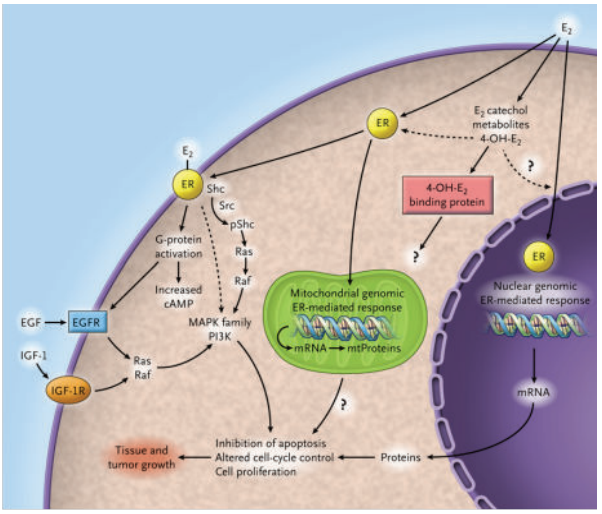

Figure 8. Estrogen receptor–signalling Pathways associated with increased proliferation and inhibition of apoptosis. In the classic mechanism of direct action, estrogens bind to ERs in the nucleus, causing dimerization with activation of the receptor transcriptional domain[97] and the subsequent binding to estrogen–response elements[98] of the target genes. The presence of ERs has also been confirmed in other cellular compartments, such as plasma membrane and mitochondria[99,100].

Some evidences indicate that estrogens inhibit the early stages of apoptosis through signalling pathways involving membrane and mitochondrial ERs[101]. In addition to the genomic actions of ER, plasma membrane–associated ERs can mediate the activation of multiple signalling cascades as: phospholipase C/protein kinase C[102], Ras/Raf/MAPK[103], phosphatidyl inositol 3 kinase/AKT[104], and cAMP/protein kinase A[105]. These non–genomic effects facilitate cross–talk between the membrane ER–signalling process and multiple signal–transduction pathways. Extracted from: Yager et al. (New England Journal of Medicine, 2006)[106].

Two distinct types of signalling can be mediated, often referred to as genomic and non–genomic pathways. In either case, the final result is the alteration of gene expression emphasizing the effects of increased proliferation and inhibition of apoptosis[14,107].

4.4.2. Products of estrogen metabolism

ER–mediated processes are considered epigenetic carcinogens: they do not play the critical role in cancer initiation because the hypothetical mutations obtained are random, but instead, they stimulate abnormal cell proliferation, a process that can lead to carcinogenesis. However, the discovery that specific oxidative metabolites of estrogens can react with DNA, supports the hypothesis that estrogens can become endogenous carcinogens by generating the mutations leading to abnormal proliferations and, therefore, to the initiation of cancer[108].

Estrogen metabolism in humans comprises two phases. Phase I, in which estrogen and estrone are oxidized by several CYP enzymes and Phase II, the detoxification pathways including sulfation, methylation and reaction with glutathione. Under normal conditions, these processes are characterized by homeostasis, a balanced set of activating and protective enzymes in which

the carcinogenic metabolites of estrogens are not available to react with DNA. However, a variety of endogenous and exogenous factors can disrupt estrogen homeostasis. The elevation of estrogens by overexpression of *CYP19A1*[109], an increase in 4-OH pathway by overexpression of *CYP1B1*[110] as well as low levels of *COMT* activity[111] could behind this dysregulation.

5. BREAST CANCER THERAPIES

Over the past two decades, breast cancer treatment has evolved to a more effective and targeted directed approach, completely transforming the disease. For many years, the medical treatment of breast cancer was reliant solely on cytotoxic chemotherapy. Currently, advances in understanding tumour biology have led to the development and approval of many novel agents that have changed the landscape of therapy. This trend is expected to continue, since agents that use novel approaches are constantly being tested. Patient outcomes will improve along with the advent of personalized medicine.

Breast cancer treatments can be classified depending on when the treatment is administered as either neoadjuvant (pre–surgery) or adjuvant (post–surgery). The neoadjuvant approach to breast cancer is used in the management of patients with high–risk breast cancers, large tumours and for locally advanced disease. Such treatment offers several clinical advantages. First, it is able to reduce the size of primary tumour in order to increase the likelihood of breast conservation rather than mastectomy[112]. Second, neoadjuvant therapy allows for the *in vivo* assessment of tumour response to the treatment, thus saving patient exposure to potentially toxic therapy. In fact, more recently, the neoadjuvant strategy has become recognized as an *in vivo* potential platform to explore the efficacy of new therapeutic agents[113].

On the other hand, the goal of adjuvant systemic therapy is eradicating micrometastasis, that is, clinically occult tumours that are present after

surgery, with a potential to metastasize. Nearly 30% of women with cancer confined to the breast and 75% of women with nodal involvement will ultimately relapse[114].

5.1. SURGERY

In early breast cancer, surgery can remove any disease that has been detected in or around the breast or regional lymph nodes. In breast conserving surgery, only the part of the breast containing the tumour is removed. By contrast, during mastectomy the entire breast is removed. Lymph nodes can also be removed either on the same surgery or as a separate operation.

5.2. RADIOTHERAPY

Radiotherapy is a highly targeted and effective treatment based on high doses of ionizing radiation. After surgery, radiotherapy is the most effective curative treatment for cancer. Given as adjuvant treatment after breast conservative surgery or even after mastectomy, radiotherapy can produce a substantial reduction in the risk of recurrence[113,115].

5.3. CHEMOTHERAPY

Chemotherapy is a systemic treatment that uses alone or combined cytotoxic chemical substances in order to prevent cell division and therefore slowing tumour growth. It can be administered orally or intravenously. Chemotherapy is usually used as an adjuvant treatment after surgery, but it also can be used before surgery. Chemotherapy has made significant progress with several landmark studies identifying clear survival benefits[116].

5.4. BIOLOGICAL THERAPY

Biological therapy is the most recently developed treatment strategy, subsequent to new knowledge regarding signalling transduction pathways in breast cancer. Some of the most important specific-targeted drugs for treating breast cancer are monoclonal antibodies or tyrosine kinase inhibitors. The monoclonal antibody trastuzumab targets the extracellular domain of the HER2 protein, blocking its dimerization and with it the downstream signalling pathways that lead to cell growth, survival and cell differentiation[117]. The drug is now regarded as one option for standard therapy in HER2-overexpressing metastatic breast cancers. Data from several studies has demonstrated that trastuzumab has contributed to reduce the rates of breast cancer mortality and recurrence[118,119].

5.5. ENDOCRINE THERAPY

The landscape of breast cancer changed dramatically with the introduction of endocrine/hormonal therapies. As the growth of certain breast cancers depends on estrogen, it might, therefore, be expected that if the source of estrogen is removed or if estrogen is prevented from binding to its receptors, tumour growth could potentially be prevented. Thus, endocrine therapy has become a pivotal treatment for women with ER-positive tumours. Multiple forms of hormone therapy currently exist.

5.5.1. Surgical Oophorectomy

Surgical oophorectomy – in other words, surgical removal of ovaries – is the oldest form of hormone therapy[65]. It causes an immediate and permanent decrease in estrogen levels. Several studies have shown the

benefits of oophorectomy in terms of disease-free survival[120]. However, the procedure has many disadvantages, including the morbidity and mortality[121] as well as its irreversibility.

5.5.2. LH-RH (Luteinizing Hormone-Releasing Hormone) Analogues

As with surgical oophorectomy, the use of LH-RH analogues (e.g. goserelin, leuprolide, and buserelin) is confined to pre- or perimenopausal women. These compounds act on the hypothalamic-pituitary axis, decreasing LH and suppressing ovarian function and therefore estrogen levels[122]. However, in contrast to ovarian ablation, this decrease is potentially reversible and normal ovarian function may return when treatment is stopped. These compounds have also demonstrated to improve long-term survival in breast cancer patients[123].

5.5.3. Selective estrogen receptor modulators (SERMs) and downregulators (SERDs)

SERMs (also known as anti-estrogens) are a unique class of therapeutic agents that act as competitive inhibitors of estrogen binding to ERs. Upon binding to hormone, the ligand binding domain (LBD) of the ER undergoes a conformational change, in order to facilitate the union of cofactors (co-activators or co-repressors) required for ER-mediated gene regulation. These cofactors are exquisitely sensitive to LBD changes[124]. SERMs generate an abnormal receptor conformation, disrupting co-activator binding to the LBD[125]. Subsequently co-repressor molecules are recruited to the ER, holding it in an inactive state[126].

However, an individual SERM can behave as an ER agonist in one tissue and

as an antagonist in another, generating a complex array of tissue–specific effects. Not only that, but they can have estrogenic effects on certain genes, even in tissues in which its predominant activity is anti–estrogenic. Although the exact mechanism behind the mixed effects of SERMs is not yet fully understood, the variable interaction with cofactors is thought to be involved. For this reason, they are termed selective estrogen receptor modulators.

Since its approval by the Food and Drug Administration in 1977[127], the SERM tamoxifen has become the most widely endocrine treatment for breast cancer. Tamoxifen inhibits the expression of estrogen–regulated genes involved in stimulating breast cancer cell growth and progression, including growth and angiogenic factors secreted by the tumour[128]. The net result is the tumour regression by a block of the cell cycle in the G1 phase[129] and, perhaps, a slightly increased rate of apoptosis[130]. Tamoxifen has been established as an effective therapy for patients with all stages of hormone receptor–positive breast cancer[128], achieving reductions in breast cancer recurrence and contralateral breast cancer of 40–50%[131]. Furthermore, it can be used as a breast cancer preventive, with notable decreases between 16% and 70% in breast cancer incidence[132,133].

Ancillary benefits can also be derived from the partial agonist properties of tamoxifen. Among the most remarkable ones are the protection against menopausal bone loss[134] and cardiovascular disease, although the data for the latter are still controversial[135,136]. However, this partial agonist activity is not released from serious adverse effects, as for example its carcinogenic potential. An alarming case, is the endometrial cancer, for which increases similar to those reported in estrogen replacement therapy have been

observed[137].

Fulvestrant is the only SERD clinically available and it is currently approved in the United States for patients with metastatic breast cancer whose disease has progressed on antiestrogen therapy. Fulvestrant binds to the ER with greater affinity than tamoxifen, resulting in marked downregulation of both the ER and PR, not only on breast tissue but also on the endometrium and bone. Fulvestrant must be given in intramuscular injections, which may limit its usage.

5.5.4. Aromatase Inhibitors (AI)

Tamoxifen provided the mainstay of endocrine therapy for many years. However, the development of AI has brought an alternative strategy for first–line managing hormone–positive breast cancer in postmenopausal women. Given that the main source of estrogen production in postmenopausal women comes from the peripheral conversion by aromatase, inhibition of this particular enzyme results in the significant further reduction of estrogen. AI administration triggers a compensatory response, with dramatic increases in gonadotropin secretion. In premenopausal women (still having large amount of aromatase substrate present in the ovary) gonadotropins stimulate ovarian follicles[138], making AI less effective in inhibiting ovarian estrogen production. Thus, in premenopausal women, AI use is restricted to special circumstances, such as prior tamoxifen failure. When this is the case, these agents must be used in combination with surgical or medical ovarian ablation[139].

Nowadays, a considerable number of AI exist, representing several generations of evolution, each of them achieving increased specificity and

greater potency. The first AI to be evaluated, such as testolactone, were steroidal inhibitors, analogues of the natural substrate androstenedione[140]. They compete with the natural substrate for the binding site and therefore, high concentrations of drug were required to maintain the inhibition. Moreover, androgenic effects were usually derived from their steroidal nature.

The starting point in the development of competitive nonsteroidal AI was aminoglutethimide, an inhibitor of several steroidogenic CYPs, including aromatase. The drug demonstrated clinical efficacy for the treatment of postmenopausal breast cancer[141]. However, the concomitant inhibition of other steroidogenic enzymes implied the need of corticosteroid substitution.

The second-generation AI include formestane and fadrozole which were associated with fewer side effects compared with standard treatment regimens at that time. However, their anti-tumour effects were not superior than those of aminoglutethimide or tamoxifen[142]. Moreover, formestane has the disadvantage of requiring intramuscular injection, and fadrozole also causes aldosterone suppression. None of these compounds are in clinical use any longer.

Third generation AI, with superior toxicity profile and convenience of administration (excellent oral bioavailability in once a day dosing), have superseded first and second generation compounds in the treatment of breast cancer. This group of agents cluster type I and type II AI. Type I inhibitors of third generation are steroidal analogues of androstenedione, but unlike testololactone, they bind irreversibly to aromatase because of its conversion by the enzyme to reactive alkylating species[143]. This process

generates long-term and specific effects, independent of continued presence of the drug, so the duration of inhibitory effects is primarily dependent on the rate of *de novo* synthesis of aromatase. These types of inhibitors include exemestane. Type II Inhibitors, by contrast, are non-steroidal and bind reversibly to the aromatase enzyme. They prevent the union of androgens by saturating the binding site. This particular subtype currently includes anastrozole and letrozole[144]. All of these third-generation AI inhibit aromatase activity by more than 98% and are more potent than earlier drug entities[145,146].

As other endocrine treatments, AI can be applied as a preventive, neoadjuvant or adjuvant manner. The findings from major studies evaluating third-generation AI, can be summarized in two treatment approaches. The first is to compare AI with tamoxifen monotherapies and the second is to evaluate the sequential treatment, in which 2–3 years of tamoxifen are followed by an AI. The rationale behind the sequential treatment was to increase the efficacy of the two agents, while reducing toxicities and preventing acquired resistance. A recent meta-analysis combining 9 trials randomizing patients between AI and tamoxifen found reduced recurrence rates with AI compared with tamoxifen[147]. Moreover, the longer follow-up of this meta-analysis allowed establishing that both breast cancer mortality and all-cause mortality are also reduced. It has been inferred from the results that 5 years of AI compared with no endocrine therapy, would reduce breast cancer recurrence by about 2/3 during treatment and by about 1/3 during years 5 – 9, and would reduce breast cancer mortality rate by around 40% throughout the first decade, and perhaps beyond[147]. It has to be emphasized that the most significant

recurrence reductions were observed in the sequential treatments, reinforcing the hypothesis that AI acquire greater superiority over tamoxifen after previous exposure to tamoxifen[147].

Therefore, at this time, the panel believes that optimal adjuvant hormonal therapy for a postmenopausal woman with RE+ breast cancer should include AI either as initial therapy or after treatment with tamoxifen.

6. SECONDARY EFFECTS OF AI THERAPY

Estrogen actions are required for normal anatomical and physiological development as well as for the maintenance of health in both males and females, so much so that, aromatase deficiency was considered incompatible with life for many years. This dogma changed with the description in the world literature of several cases of aromatase deficiency in humans[148]. Clinical cases in which aromatase is inactivated because of germline mutations[148] as well as the generation of aromatase knock–out animals[149] have highlighted the important role of estrogens.

AI are generally safe drugs and are reasonably well tolerated by most patients. They have been studied in very large adjuvant trials with careful documentation of both toxicity and patient compliance. However, the emerging field of breast cancer survivorship –estimated at slightly 3.2 million women in most developed countries in 2012[150]– and clinical experience is suggesting some adverse effects that negatively impact quality of life and even persistence with therapy[151]. In fact, it has been postulated that the relative toxicity of AI versus tamoxifen[152,153] may explain the difficulties of finding overall survival benefit of AI vs. tamoxifen in postmenopausal breast cancer patients[154].

6.1. CARDIOVASCULAR RISK AND LIPID METABOLISM

The simple observation of the differential distribution of body fat between pre– and postmenopausal women gives an idea of the integral role of estrogens in lipid homeostasis and adipose tissue distribution[155,156]. Estrogens

have been postulated to induce cardioprotective effects, not only via receptors, but also influencing nitride oxide generation and bioavailability[157]. It is hardly surprising, therefore, that AI have been associated with unfavourable lipid profile in some studies[154,158]. The real concerns about this issue, fall on the association of serum lipids with cardiovascular diseases[159]. A number of meta-analysis[147,154,160–162] integrating data from 11 different randomized control trials (RCT) have found that the use of AI is associated with an increased risk of coronary heart disease in comparison with tamoxifen. However, these studies are often subject to multiple confounding variables and the results are controversial. It is not clear yet whether changes in serum lipids and cardiac events are the result of tamoxifen withdrawal rather than a direct effect of AI-therapy[163], since tamoxifen may reduce the risk of cardiovascular disease[164]. This notion has yet to be fully elucidated.

6.2. MUSCULOESKELETAL EFFECTS

A major concern with AI therapy is related to the musculoskeletal pain. Numerous patients on AI complain of severe musculoskeletal pain and joint stiffness. These symptoms have been observed at approximately 2 months after treatment starting and to peak at around the 6-months, but they can also appear up to 2 years after initiation of therapy[165]. Generally, symmetrical joint pain most commonly affecting the wrists, hands and knees as well as carpal tunnel syndrome and trigger finger are common complaints associated with these agents. Other symptoms may include morning stiffness, myalgia and decreased grip strength. Discomfort may be most noticeable on awakening and often improve with morning activities.

Patients frequently mention that they feel they have "aged" abruptly. Whatever musculoskeletal symptoms are, they have on average a modest impact on day-to-day function and activity[6–8].

Unfortunately, the absence of uniform and clear definition of the problem makes us to use the term "arthralgia" (defined as joint pain) to mean a range of symptoms that are probably broader than arthralgia alone. It is precisely this lack of a consistent description which renders impossible having an accurate assessment of the incidence of AI-related arthralgia (AIA). Thus, various RCTs have described a wide range of AIA incidence, likely because they are each defining AIA differently. Moreover, the majority of the studies reporting data on AIA were not created primarily for this purpose. Hence, AIA incidence ranges from 5% of the Intergroup Exemestane Study and 35% of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. The single study specifically designed to assess the prevalence of these symptoms, announce as much as 47% of women developing AI-related joint pain[166]. Thus, musculoskeletal toxicities become the most common side effect associated with AI-therapy.

6.2.1. Etiology of AIA

To date, the etiology of musculoskeletal pain under AI has yet to be defined. Despite many factors such as obesity, previous chemotherapy or HRT as well as the exacerbation of pre-existing myalgia and arthralgia may explain the severity of these symptoms[167,168], estrogen deprivation is inevitably involved. Ample physiological and pharmacological evidence lend much weight to this hypothesis. It is well known that the prevalence of joint and widespread pain increases progressively with age in women, reaching a

maximum in the group of 50 – 59–years[169,170]. Arthralgia following menopause has been informed in several studies, being reported by more than half of the women[171]. The majority of studies demonstrate that HRT alleviates these symptoms, although the size of the effect seems to be small. Moreover, HRT withdrawal increases the incidence of pain and stiffness[172]. Nonetheless, the question of the pathogenesis and anatomic features of AI–induced arthralgia remains to be solved.

Pain can emanate from a variety of articular structures innervated with nociceptive fibbers, including the joint capsule, synovium, periosteal bone, ligaments and even, periarticular structures. ERs have been found in human synovia and cartilage[173,174] and animal studies have shown that ovariectomy accelerates cartilage turnover, presumably due to the low–estrogen state[175]. Perhaps, this higher rate of cartilage turnover contributes to bone pain from lack of cushioning in the joints. Some studies have also identified radiological features, such as tenosynovial changes and joint effusions associated with AI–therapy[176,177]. These radiologic evidences may have an inflammatory nature. The effects on inflammation within the joint are not well known but it is already documented that higher levels of estrogen suppress inflammatory cytokine production, and lower estrogen levels increase their production. Thus, for example, women who are beginning the menopause have higher levels of inflammatory cytokines such as interleukin (IL)–1 and tumour necrosis factor–α (TNF–α). These cytokines may contribute to both the postmenopausal syndrome of arthralgia and to AIA. The conversion of androgens into estrogens in synovial cells, by aromatase, is accompanied by IL–6 reduction[178]. Thus, AI are thought to increase IL–6, and subsequently the pro–inflammatory response. Even more relevant may

be the antinociceptive effects of estrogen. Estrogen has direct effects on opioid pain fibbers in the spinal cord and brain, which have been found to express ERs[179]. In some species, aromatase has been found in dorsal horn cells[180], in which the conversion of androgens could provide a source of estrogen in the spinal cord. During arthralgia, nociceptive neurons acquire a heightened sensitivity either at the joint itself or centrally[181]. Consequently, the processing of nociceptive input from the joint is amplified, leading to enhanced responses to innocuous stimuli and a perpetuation of the feeling of pain. In addition, inflammatory episodes induced by estrogen depletion could account for enhanced nociception[182]. Recently, the transient receptor potential ankyrin 1 channel, a polymodal sensor, has been postulated to be a mediator of the proinflammatory/proalgesic actions of AI [183].

6.2.2. AIA significance: adherence, treatment efficacy and mortality.

Lack of adherence to prescribed medications is a well–known problem in the medical literature[184]. Many patients fail to fill the initial prescription (non–initiation), to take the drug on a daily basis as prescribed (nonadherence), or to continue long–term with the drug (early discontinuation). Such departures from optimal drug use, frequently result in treatment failure[184].

Several studies have found superior non–adherence and discontinuation rates for AI than those for tamoxifen[185]. It has been estimated that more than 25% of patients do not adhere to prescribed therapy with AI[185–189]. Evaluation of both non–adherence and discontinuation showed that only 50% of patients on AI took adjuvant hormonal therapy for the full duration at the optimal schedule[188].

Although a number of risk factors have been associated with non–

adherence, such as comorbidity, age, prior treatments, pre-existing pain, alcohol consumption, smoking and cancer at a non-curable stage[151,187,188,190], none is an absolute predictor. On the other hand, AI-associated toxicities are a major barrier to the full application of effective treatment. Specifically, joint pain is so troubling that it is actually responsible for an important part of withdrawals[191].

AIA carries further significance beyond quality of life and compliance issues for breast cancer survivors; It may also be tied to recurrence risk: women who developed arthralgia during AI therapy actually had a lower risk of breast cancer recurrence and survival[192–194]. One could argue that the appearance of symptoms could lead to lower adherence and therefore lower subsequent efficacy[195,196]. However, these symptoms are believed to be related to lowered estrogen concentrations: that is, women with arthralgia could simply have more effective estrogen depletion on AI, leading to a lower risk of recurrence. Or, perhaps the arthralgia is mediated by a totally different mechanism, which may also have antitumor properties.

6.2.3. AIA management

The current management of AIA should involve patient education before beginning AI therapy that joint pain is a very common side-effect[197]. The most effective management option is AI discontinuation with prompt resolution of symptoms. Although there are still no clear evidences to state that switching to another AI can be beneficial, one trial showed that women who did not tolerate one AI because of arthralgia were able to tolerate another AI instead[198]. Similarly, switching patients to tamoxifen may also provide significant benefit[199]. Therapeutic options include the use of non-

steroidal anti-inflammatory drugs, cyclo-oxygenase-2 inhibitors, antidepressants and gabapentin, each associated with their own unwanted effects. Some patients may even require surgery[200]. However, a combination of lifestyle changes, such as introducing weight-bearing exercise and yoga, acupuncture, abstaining from smoking and being moderate in alcohol consumption should also be recommended[166,201,202].

In any case, it is clear that musculoskeletal discomfort in breast cancer patients treated with AI have been underestimated and that these symptoms can be severe, debilitating, and can limit compliance.

6.3. OSTEOPOROSIS AND RISK OF FRACTURE

The word of osteoporosis literally means porous bone (**Fig. 9**). Osteoporosis is a bone disease characterized by a decrease in bone mass and microarchitectural alterations which results in bone fragility and increased risk of fracture[203].

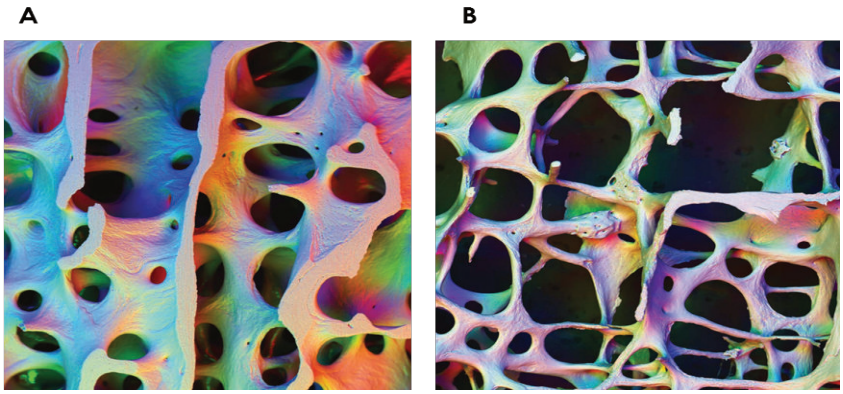

Figure 9. Electron microscope images of normal (A) and osteoporotic bone (B). (A) Normal bone architecture: strong, interconnected plates of bone are visible. (B) Osteoporotic architecture: the bone is heavily eroded in places by the action of

osteoclasts and consists mainly of thin, fragile struts, weakening the mechanical properties of the bone. Adapted from: Boyde et al. (Endocrine, 2002)[204].

Bone mass, bone density or bone mineral density (BMD) refers to the amount of mineral in bone tissue. Clinically it is measured by proxy, according to optical density per square centimetre of bone surface, determined by dual-energy X-ray absorptiometry (DXA)[205]. The T-score is the primary outcome of a densitometry, indicating the number of standard deviations above or below the BMD mean of young healthy adults. It classifies patients into three diagnostic categories: normal BMD, low bone mass (osteopenia), or osteoporosis. According to the World Health Organization, osteoporosis is defined as a T-score below -2.5.

It is calculated that osteoporosis affects 200 million women worldwide. However, the public health and clinical importance of osteoporosis lies in the fractures associated with the disease. Osteoporotic fractures (fragility fractures, low-trauma fractures) are those occurring from mechanical forces, without major trauma. Typical fractures in patients with osteoporosis include vertebral (spine), proximal femur (hip), wrist and proximal humerus[206]. Osteoporosis is estimated to cause more than 8.9 million fractures annually, resulting in an osteoporotic fracture every 3 seconds. The lifetime of patients with osteoporotic fracture decreases considerably, potentially rising 40% for white women[207]. Furthermore, 50% of women with osteoporotic hip fractures develop disability, with significant impact on the capacity to live independently and, in most cases, institutionalization. This morbidity burden has considerable medical, social and financial implications.

Osteoporosis may be either a primary or a secondary form. Primary osteoporosis is the more common form and it is associated with the process of normal aging. The skeleton acquires the maximal bone density ("peak bone mass") at 25–30 years. Thereafter at about 30 years, a negative bone balance sets in, so that on average 1% of bone is lost every year. Moreover, the rate of bone loss accelerates during postmenopausal period due to the steroid declining. Several risk factors have been associated to primary osteoporosis, notably among them; advancing age, low BMI, family history of osteoporotic fractures, early menopause, sedentary lifestyle, excessive alcohol, low calcium and/or vitamin D intake as well as inadequate sun exposure.

On the other hand, secondary osteoporosis occurs as a result of certain medical conditions, such as endocrine and metabolism disorders (e.g., hypogonadism, hypercortisolism, hyperparathyroidism, hyperthyroidism, anorexia), lymphoproliferative disorders, intestinal malabsorption conditions, rheumatoid arthritis, renal failure and collagenopathies. Certain drugs can also contribute to the development of secondary osteoporosis, such as corticosteroids, selective serotonin reuptake inhibitors, anticoagulants, antidiabetic medications and, in this particular case, AI.

6.3.1. Bone structure

Bone is a porous mineralized structure made up of cells, vessels, and crystals of calcium compounds. It provides support and protection of vital internal organs and bone marrow as well as the muscle attachment for locomotion. Bone also plays a central role by contributing to life–supporting metabolic exchange, serving as a reserve of calcium and phosphate needed for the

maintenance of serum homeostasis.

The structural components of bone consist of extracellular matrix and cells. Two types of bone are observed in the normal, mature human skeleton: cortical and trabecular[203]. Although macroscopically and microscopically different (**Fig. 10**), the two forms are identical in their chemical composition.

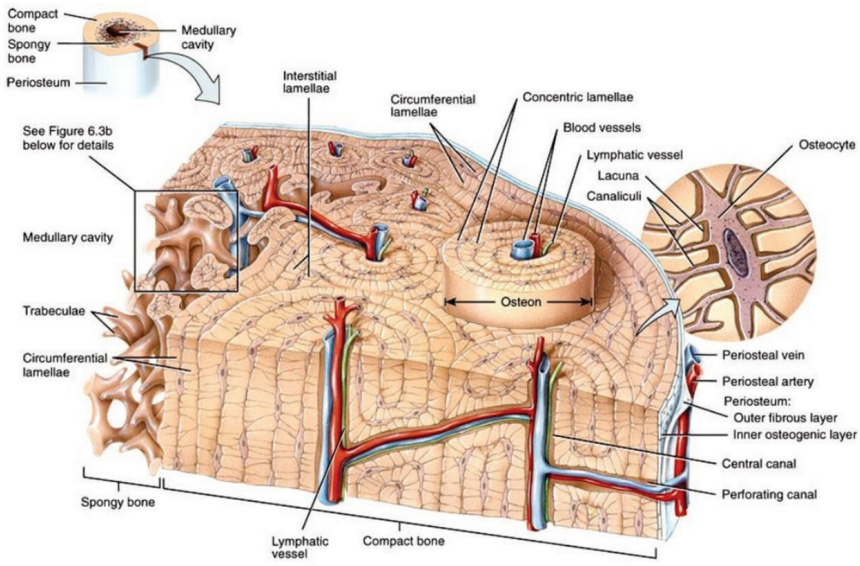

Figure 10. Anatomy of cortical and trabecular bone. Extracted from: http://classroom.sdmesa.edu/eschmid/Chapter6-Zoo145.htm

Cortical bone, which comprises 80% of the total bone mass of an adult skeleton, constitutes the outer layer of all skeletal structures. The major part of the cortical bone is calcified and has a slow turnover rate. It is dense and compact, thus having a high resistance to bending and torsion. Its function is to provide mechanical strength and protection. It can also participate in metabolic responses, particularly when there is severe or prolonged mineral deficit. Trabecular or cancellous bone is a network of

internal plates and rods forming a 3D branching lattice interspersed in the bone marrow compartment. It is found principally at the metaphysis and epiphysis of long bones and in cuboid bones, such as the vertebrae. It represents 20% of the skeletal mass but has nearly ten times the surface area of compact bone. Trabecular bone is less dense, more elastic and has a higher turnover rate than cortical bone, exhibiting a major metabolic function. It contributes to mechanical support and provides the initial supplies of mineral in acute deficiency states.

6.3.2. Bone matrix

Similar to other connective tissues, bone cells are not the primary constituents of bone by weight. Rather, the extracellular matrix (ECM) represents approximately 90% of the organic composition of the whole bone tissue. The mineralized portion of the ECM is composed largely of calcium–phosphate in the form of hydroxyapatite with small amounts of carbonate, magnesium, and acid phosphate.

The organic component (osteoid) consists primarily of type–I collagen, and to a lesser extent of non–collagenous proteins, including proteoglycans, osteocalcin, osteopontin, osteonectin and bone sialoprotein. Bone ECM determines the mechanical properties of the skeleton. The mineralized portion of the ECM imparts rigidity and load–bearing strength to the material, while the osteoid provide plasticity[208]. Furthermore, the diverse array of ECM proteins support various biological cell functions[208]. This capacity is largely determined by their ability to bind multiple interacting partners such as other ECM proteins, growth factors, signal receptors and adhesion molecules[208].

6.3.3. Osteoblasts

Osteoblasts originate from pluripotent mesenchymal stem cells, which have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes, myoblasts, or fibroblasts[209]. Commitment of mesenchymal stem cells to the osteoblast lineage requires the complex integration of bone morphogenetic proteins, Wnt, Notch, Hh, and fibroblast growth factor (FGF) pathways. The transcription factor Runx–2 seems to be a potential focal point for signalling integration[210]. The primary function of these cells is the production of the bone matrix, contributing to expansion of bone volume by laying down osteoid and secreting factors that facilitate mineral deposition. Moreover, osteoblasts regulate the differentiation of osteoclasts[211] and bone resorption activity by different mechanisms[212].

Toward the end of the matrix–secreting period, 15% of mature osteoblasts are entrapped in their own bone matrix and differentiate into osteocytes. Alternatively, on a quiescent bone surface, the osteoblast can develop into a flattened bone–lining cell of a single layer forming the endosteum against the marrow and underlying the periosteum directly on mineralized surfaces.

6.3.4. Osteocytes

Osteocytes represent terminally differentiated osteoblasts and account for about 95% of the whole cell population in the mature bone tissue. From each osteocyte cell body, an extensive filopodia processes originate and radiate through the mineralized matrix via spaces called the canaliculi. This filopodia radiate in different directions and form an intricate intercellular network connecting them with each other and with the bone surface lining cells and osteoblasts[213]. Osteocytes are the pivotal cells orchestrating the

biomechanical regulation of bone mass and structure for efficient load bearing. They transduce stress signals from bending or stretching of bone into biologic activity, leading to adequate bone mass and architecture[214].

6.3.5. Osteoclasts

Osteoclasts, the exclusive cells with ability to resorb bone, are multinucleated giant cells derived from mononuclear precursors of the monocyte–macrophage lineage of bone marrow[211]. Receptor activator of nuclear factor–κβ (RANK–L) and macrophage colony stimulating factor (M–CSF) are the two critical and sufficient cytokines for basal osteoclastogenesis. Both RANK–L and M–CSF are produced mainly by cells of the osteoblastic line, making the presence of these cell types, indispensable for the physiological recruitment of osteoclasts from their precursors[211]. RANK–L binds to its receptor, RANK, on the surface of osteoclasts precursors and it is critical for osteoclast formation[215]. M–CSF contributes to the proliferation, survival and differentiation of osteoclast precursors[216]. Osteoprotegerin (OPG), also secreted by osteoblasts and osteogenic stromal stem cells, protects the skeleton from excessive bone resorption by binding to RANK–L with high affinity and preventing it from interacting with RANK, and hence favours increased bone mass[217].

6.3.6. Bone remodelling

To maintain a healthy skeleton with optimal mechanical integrity, bone undergoes the remodelling process throughout life. This remodelling, in which aging or damaged bone is gradually replaced by new tissue, is an integral part of the calcium homeostatic system and provides a crucial mechanism for adaptation to physical stress[218,219]. This is accomplished

through the carefully orchestrated collaboration among several types of bone cells, combined into defined anatomical spaces termed basic multicellular units (BMUs). A remodelling cycle might begin with osteocytes recognizing that a specific area of bone needs to be replaced[220] and signalling through their canaliculae to surface cells (**Fig. 11**). The consequence is the separation of lining cells from the underlying bone and the recruitment of osteoclasts and osteoblasts to generate a new BMU[221]. As the entire BMU moves forward, osteoclasts resorb bone and die by apoptosis. If new osteoclasts are not formed, resorption ceases.

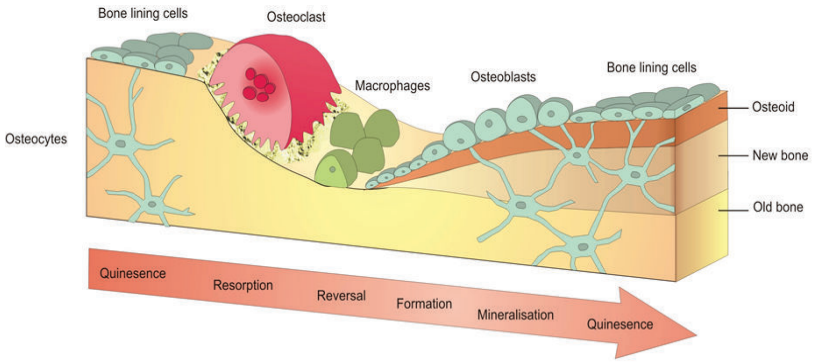

Figure 11. The bone remodelling process. Old bone is resorbed by osteoclasts. Following resorption, macrophage–like cells are found at the remodelling site. Osteoblast precursors are then recruited, which proliferate and differentiate into mature osteoblasts, before secreting new osteoid. The matrix then mineralizes to generate new bone, thus completing the remodelling process. Extracted from: http://www.york.ac.uk/res/bonefromblood/background/boneremodelling.html

Once the resorption phase is terminated and after a brief reversal phase[222], a team of osteoblasts is recruited, laying down bone until the resorption cavity is completely filled. During this process, the osteoblasts become progressively flatter and wider. At the end of the bone–forming phase some

of them become embedded in the bone as osteocytes, some die by apoptosis and those remaining when the process is completed become the lining cells that cover the new quiescent surface[223]. The net result is the replacement of a packet of old bone with new bone (**Fig. 11**). Pathological imbalance in these cellular processes can lead to disease conditions of bone loss, in particular osteoporosis.

6.3.7. Molecular effects of estrogen deficiency on remodelling

An essential requirement for balanced remodelling in adults is that resorption and formation are balanced, so that old bone is continuously replaced at the exact amount removed by resorption. Thus, tight control of bone remodelling at the level of the BMU is essential to maintain structural integrity. The regulation of bone remodelling is complex, being controlled by hormones and many other proteins both at systemic and local level. Parathyroid hormone (PTH), vitamin D, calcitonin, the growth hormone/insulin growth factor (IGF)–1[224] system and IGF–2[225], thyroid hormones[226], glucocorticoids[227], FSH[228] and serotonin[229] are among the most important factors influencing systemic regulation of bone cell functions.

Estrogens also play a key role in maintaining both normal bone turnover and bone mass. Therefore, long–term estrogen deprivation is associated with the development of osteoporosis[230] and increased risk of bone fracture. The mechanisms whereby loss of estrogen increase bone turnover are complex and multifaceted, but broadly speaking, new osteoclast formation with subsequent increase of BMUs, is involved[231]. Such activation expands the remodelling space, increases cortical porosity and enlarges the resorption area on trabecular surfaces in a cytokine–driven process[232] (**Fig. 12**).

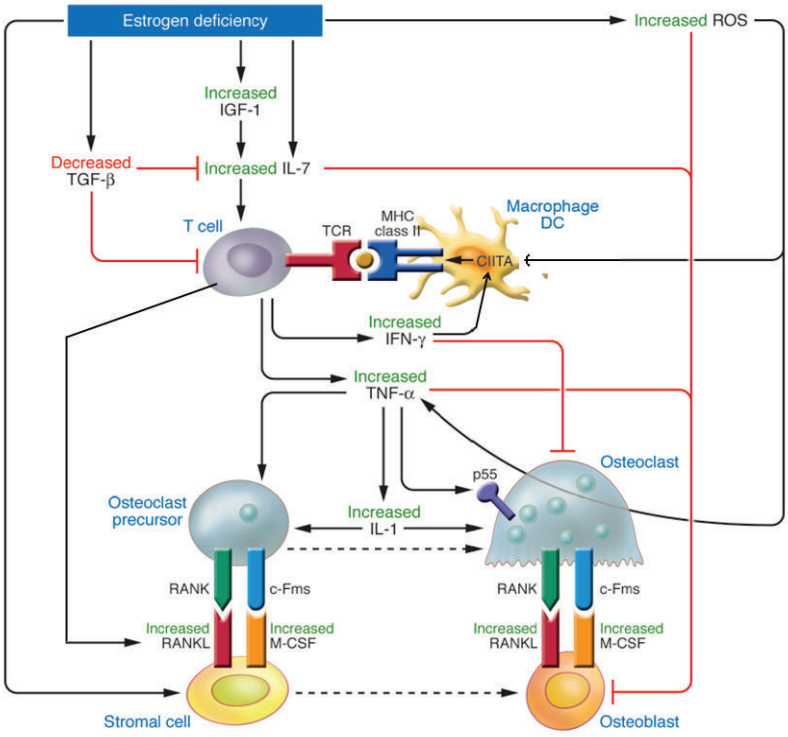

Figure 12. Schematic representation of the main mechanisms by which estrogen deficiency leads to bone loss. The bone loss induced by estrogen deficiency is due to a complex interplay of hormones and cytokines that converge to disrupt the process of bone remodelling. Adapted from: Weitzmann et al. (The Journal of Clinical Investigation, 2006)[233].

Estrogen deficiency leads to an increase of T-cell activation, proliferation and lifespan by several mechanisms including, antigen presentation, transforming growth factor beta (TGF-β) stimulation and IL-7 increasing. Once activated, T-cells, in turn, contribute to osteoclast proliferation and activation by means of cytokine production[234]. Molecules such as TNF- α, interferon gamma (INF-ɣ), IL-1 as well as the RANK-L itself act synergistically in order to influence osteoclast formation, lifespan and activity[234-239]. Osteoclast apoptosis is also inhibited under estrogen

deprivation[238,240]. In parallel to this phenomenon, the coupling process elicits a compensatory increase in bone formation. However, in spite of stimulated osteoblastogenesis, the rate of bone formation is inadequate to compensate for enhanced bone resorption and consequently, increased bone loss is the net effect. Augmentation of osteoblasts and osteocyte apoptosis and limitation of the activity of mature osteoblasts are among the factors mediated by estrogen depletion to limit the magnitude of the compensatory response[241,242]. As we have seen, the relationship among estrogen, the immune system and the skeleton is widely documented.

6.3.8. Aromatase inhibitor induced bone loss (AIBL) and fracture risk.

As previously stated, extragonadal synthesis of estrogen plays an important role in postmenopausal women. In human, one of the extragonadal sites of estrogen biosynthesis is bone. Aromatase has been found in bone cells and its activity is comparable to that present in adipose stromal cells[243]. Thus, local aromatase expression in bone could be the major source of estrogen responsible for the maintenance of mineralization[89].

Considering the important role of estrogens in bone, it seems logical, therefore, that the profound estrogen suppression induced by AI–treatment (exceeding the gradual decrease seen in healthy menopausal women[244]), leads to some unfavourable effects on this compartment; AIBL at the lumbar spine (LS) and hip, reported in a number of RCTs vary from 1.7% to 5.8% per year[245–248]. On average, the rate of AIBL is estimated at 2.6% annually, surpassing the bone loss of 1% per year observed in healthy postmenopausal women. Overall, fracture risk is increased a 47% with AI use compared with tamoxifen therapy[154]. Fractures are increasingly recognized

as important clinical issues for breast cancer patients since they are associated with chronic pain, loss of mobility and even shorter survival. An understanding of AIBL is critical for determining how to assess the risk and identifying which patients may benefit from preventive therapy.

7. AIBL MANAGEMENT

Preserving and/or restoring bone health is an important component of modern breast cancer management. All women diagnosed with the disease should be encouraged to follow the osteoporosis screening and prevention guidelines of the general population.

7.1. LIFESTYLE INTERVENTIONS

Lifestyle choices and dietary habits provide the necessary framework, improving not only bone health but overall health as well. Patients should be advised to limit alcohol intake, avoid excess caffeine, quit smoking and follow a well–balanced diet. Adequate dietary calcium and vitamin D are also critical, requiring supplements when appropriate[249,250]. In addition, patients should also be encouraged to participate in weight–bearing physical activity to increase bone density and muscle strength[249].

7.2. BONE LOSS SCREENING AND MONITORING

Close monitoring and consideration of proactive measures, including drug therapy when necessary, to preserve bone health are encouraged for postmenopausal patients with breast cancer on AI treatment[251]. Thus, in patients initiating AI, DXA of the hip and LS at baseline and annually thereafter is advised. However, available data suggest that BMD measurement should not be the sole criterion for determining fracture risk, but a combination of BMD and clinical risk factors, is optimal[252] (**Fig. 13**).

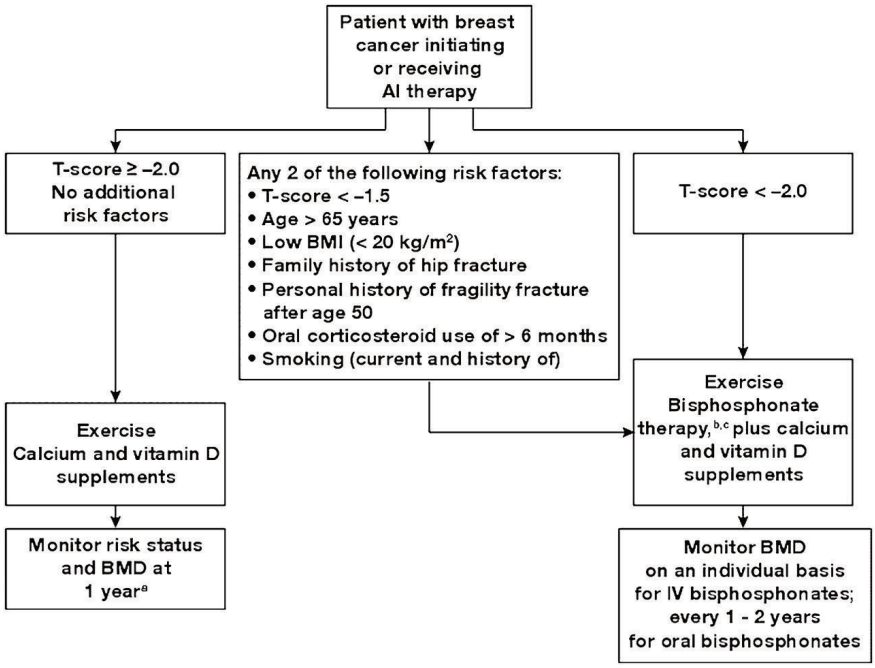

Figure 13. Recommended algorithm for managing bone health in women receiving AI therapy for breast cancer. [a]If patients experience an annual decrease in BMD of ⩾10% (use lowest T-score from three sites) secondary causes of bone loss such as vitamin D deficiency should be evaluated and antiresorptive therapy initiated. [b]Denosumab may be a potential treatment option for some patients. [c]Although osteonecrosis of the jaw is an uncommon event, regular dental care and attention to oral health is advisable in patients receiving bisphosphonates or denosumab. Adapted from Hadji et al. (Annals of Oncology, 2011)[249].

Risk factors found to increase fracture risk in women with breast cancer include AI therapy, T-score < –1.5, age > 65 years, low BMI (<20 kg/m^2), family history of hip fracture, personal history of fragility fracture after age 50, oral corticosteroid use >6 months and smoking. For the prevention of fracture in patients taking AI, those women at high risk of fracture, including patients with osteoporosis (or history of fragility fracture) and patients with osteopenia who have risks for fracture other than AI therapy[249], should

be treated with pharmacological therapy.

7.3. PHARMACOLOGICAL THERAPY: BISPHOSPHONATES (BP)

Although there is currently no approved treatment or any prevention therapy for the AIBL, clinical trial evidence indicates both BP and denosumab are effective in maintaining bone density. BP are specific inhibitors of bone resorption[253] and they are considered the first line pharmacologic therapy for the management of osteoporosis and other diseases of high bone turnover[254]. BPs' pronounced affinity for bone, relative to other tissues, enables them to attain a high local concentration throughout the entire skeleton, achieving highly specific interaction with the relevant cellular sites of action. This critical pharmacological feature makes them the ideal candidates for treatment of skeletal disorders. ASCO guidelines for addressing bone health issues in women with breast cancer[251], as well as the osteoporosis guidelines[255], rely on BMD measurements as the key indicator for therapy. In general, these guidelines recommend BP therapy (alendronate, risedronate and zoledronic acid) when BMD T–scores have dropped into (or near) the osteoporotic range[251,255]. However, earlier intervention may be beneficial to minimize the effects on the skeleton and preserve patient quality of life and functional mobility.

7.3.1. Mechanism of action

Structurally, BP are stable analogues of inorganic pyrophosphate (PPi), a naturally occurring compound released as a by–product of many intracellular metabolic reactions. PPi is an endogenous regulator of bone mineralization capable of inhibiting calcification by binding to hydroxyapatite crystals[256]. Like their natural analogue PPi, BP also bind to

hydroxyapatite crystals, but the carbon atom replacing the oxygen atom renders the molecule resistant to biological degradation.

The nitrogen-containing BP (e.g., pamidronate, alendronate, ibandronate, risedronate and zoledronate) act by inhibiting farnesyl pyrophosphate synthase[257], a key regulatory enzyme in the mevalonate pathway (**Fig. 14**). Posttranslational prenylation of small GTPases is essential for the regulation of core osteoclast cellular activities, including stress fibber assembly, membrane ruffling and survival[258,259].

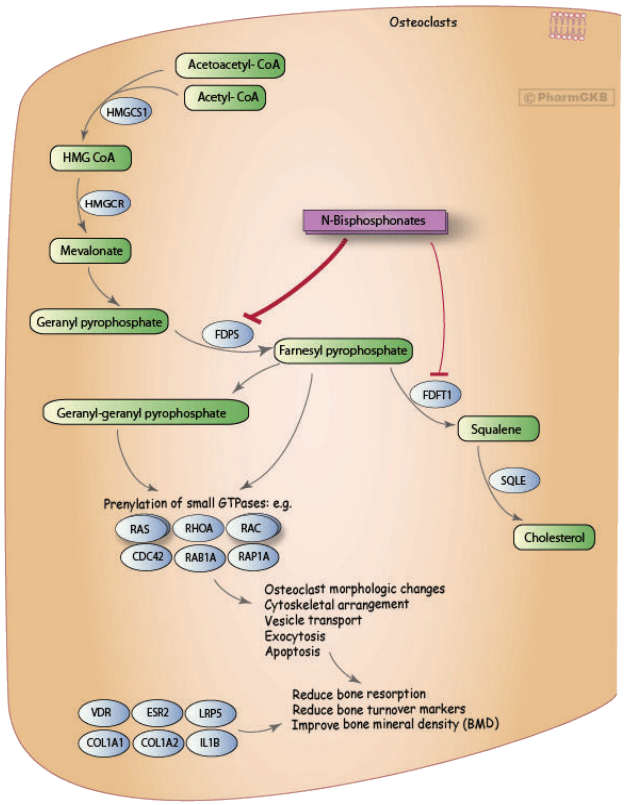

Figure 14. BP pathway: mechanism of action of nitrogen-containing BP in osteoclasts. Adapted from: Gong et al (Pharmacogenetics and genomics, 2011)[260].

Therefore, inhibition of these reactions leads to osteoclast apoptosis[261,262]. Moreover, BP also cause changes in osteoclast morphology[263,264], culminating in a decreased activity[265]. Furthermore, it has been suggested that BP also function to limit both osteoblast and osteocyte apoptosis[266]

7.3.2. Clinical role of BP

BP have emerged as the leading effective treatment for postmenopausal and other forms of osteoporosis. Either oral (etidronate[267], risedronate[268], ibandronate[269] and alendronate[270]) or intravenous (zoledronate[271]) BP are acceptable options. All have been approved as therapies in many countries, to prevent or reduce postmenopausal osteoporosis[255]. They have also demonstrated efficacy in preserving BMD in several trials[248,250,272–276]. However, evidence to date is controversial about varying risk for fragility fractures[270,277,278].

In addition, BP treatment has also been associated with a reduction in the risk of skeletal morbidity in patients with bone metastasis from breast cancer[279] as well as in breast cancer recurrence[280].

7.4. FARMACOLOGICAL THERAPY: DENOSUMAB

Denosumab is a humanized monoclonal antibody against RANK–L that reduces osteoclastogenesis. It has been shown to improve BMD in postmenopausal women[281]. Denosumab can be used as initial therapy in certain patients at high risk of fracture, such as older patients who have difficulty with the dosing requirements of oral BP or who have markedly impaired renal function. In addition, denosumab is an option for patients who are intolerant or unresponsive to BP.

7.5. THE ROLE OF BONE MICROARCHITECTURE IN FRACTURE RISK: TRABECULAR BONE SCORE (TBS)

The current definition of osteoporosis reflects the changing perspective of the disease, being no longer considered as a disorder of low bone mass density alone. Measurement of BMD with DXA is so-called gold standard for diagnosis of osteoporosis, serving as a surrogate marker for the mechanical competence of bone and fracture risk[282]. However, it is subject to several constraints.

First, DXA provides a two-dimensional projection of a three-dimensional structure and hence it cannot capture bone geometry or microarchitecture. Thus, the obtained values do not represent the true volumetric BMD but rather a projected areal BMD. This causes BMD to be confounded by bone size so it cannot distinguish between increased BMD values arising from thicker bones (geometric change) than those arising from increased tissue mineral density (material change). Therefore, BMD gives no information about structural properties such as bone size, bone geometry and also microstructural properties such as trabecular orientation and cortical porosity. Moreover, the test can also be distorted by scan artefacts such as aortic calcification, soft-tissue calcification and others.

It is estimated that <50% of the variation in the whole-bone strength is attributable to variations in BMD. In fact, most individuals with a fragility fracture have BMD values in the osteopenic or even normal range[283]. Furthermore, improvements in spine BMD during treatment with antiresorptive agents accounts for a predictable but small part of the observed reduction in the risk of vertebral fracture[284]. Consequently, BMD

cannot be used as the sole predictor of bone strength.

Assessment of skeletal microstructure can be made with some techniques as histomorphometric analysis of the transiliac crest bone biopsy, high–resolution peripheral quantitative computed tomography, flat–panel volume CT and magnetic resonance imaging. However, these techniques are invasive and/or not routinely available.

TBS is a textural index that evaluates pixel grey–level variations in the lumbar spine DXA image and has been proposed as a clinical tool capable of assessing trabecular microarchitecture (**Fig. 15**). TBS can be extracted from any available DXA image, even thought it was obtained years before, without further patient inconvenience.

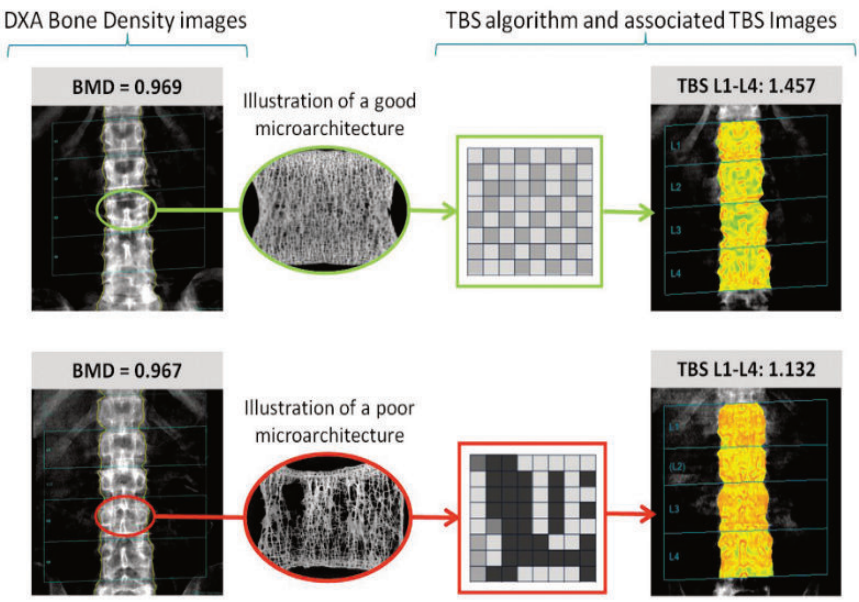

Figure 15. Representation of the TBS principles. The TBS value is derived by an algorithm that analyses the spatial organization of pixel intensity, which in turn

corresponds to the differences in the X-ray absorption power of an osteoporotic bone versus a normal trabecular pattern. Extracted from: Silva et al. (Journal of Bone Mineral Research, 2012)[285].

The dense trabecular microstructures projected onto a plane generate an image containing a large number of pixel value variations of small amplitude. Conversely, a porous trabecular structure produces an image with a low number of pixel value variations of high amplitude. A variogram of those projected images can estimate a 3D structure from the existing variations on the 2D projected images. A high TBS value is associated with better bone structure, whereas low TBS values indicate worse bone structure (**Fig. 15**).

TBS has been demonstrated to correlate with trabecular bone connectivity, trabecular number and thickness as well as space between trabeculae[286,287]. Hence, it is capable of differentiating between two 3D microarchitecture of cancellous bone with the same bone density but different trabecular characteristics[288]. TBS is a strong and BMD-independent predictor of current and future osteoporotic fractures in adults[286,289,290] and provides BMD-independent information when monitoring the effects of osteoporosis treatments. Combining BMD and TBS measurements provides more accurate assessment of osteoporotic fracture risk than does either technique alone[287].

The effects of AI on bone microarchitecture have scarcely been explored. The uncertainty about the capacity of BP to reduce fracture risk in AI-treated patients[278] triggers the idea that AI may induce changes in bone beyond BMD reduction. Generally speaking, studies on the impact of AI on

TBS report larger decreases in BMD compared with TBS[291,292]. Moreover, RCTs evaluating the skeletal preservation of BP in both healthy postmenopausal[293,294] and AI-treated[295] women do not describe remarkable improvements but rather a "positive maintenance" of bone microarchitecture.

8. VITAMIN D AND AI ADVERSE EFFECTS

Vitamin D is a complex nutrient that functions as a hormone. The associations between vitamin D concentrations and various conditions and diseases have been assessed in a large and rapidly expanding literature. Historically, vitamin D had been linked to calcium, phosphorus, bone metabolism, osteoporosis, fractures, muscle strength and falls[296]. However, in the last two decades, growing scientific attention turned to non-skeletal diseases such as cardiovascular, infectious and autoimmune diseases, metabolic disorders, as well as cancer and mortality[297].

8.1. SYNTHESIS OF VITAMIN D

Vitamin D exists in two main forms, vitamin D_3 ($VitD_3$) or cholecalciferol and vitamin D_2 ($VitD_2$) or ergocalciferol, differing in their side chain structure. In humans, the majority of $VitD_3$ is produced in the skin, from 7-dehydroxycholesterol (DHC) upon exposure to ultraviolet B radiation (**Fig. 16**). Only a small proportion is obtained from animal sources such as oily fish and egg yolk. $VitD_2$ is predominantly obtained from plant sources. Commonly, vitamin D refers collectively to $VitD_2$ and $VitD_3$[298].

Whether it is derived from the diet or synthesized in the skin, vitamin D undergoes two successive hydroxylation steps mediated by CYPs enzymes. The first step occurs in the liver, by 25-hydroxylase which converts vitamin D into 25-hydroxy vitamin D (25(OH)D). Another enzyme, the 1α-hydroxylase, converts 25(OH)D to the biologically active form of vitamin D, 1,25-dihydroxy vitamin D ($1,25(OH)_2D$ or calcitriol) in the proximal tubule of

the kidneys. This 1α–hydroxylation is under control by serum PTH and FGF–23 in response to serum calcium and phosphate and represents the rate–limiting step in the synthetic pathway[298].

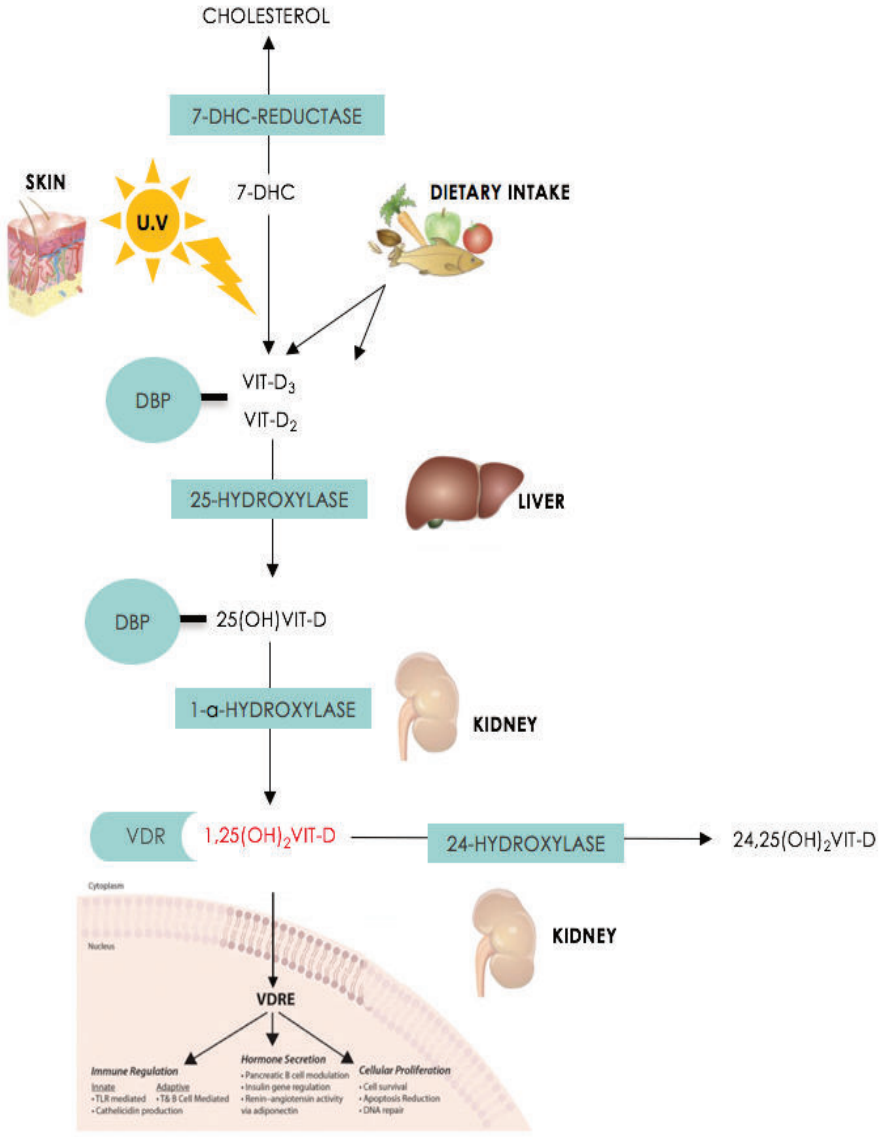

Figure 16. Vitamin D synthesis, gene activity and effects on target.

Vitamin D is transported in the circulation predominantly bound to vitamin D–binding protein (DBP) and albumin, with <1% in the free form. The active vitamin D metabolite mediates its biological effects by binding to the vitamin D receptor (VDR). VDR belongs to the nuclear receptor superfamily of steroid/thyroid hormone receptors and it is expressed by cells in most organs, including the brain, heart, skin, gonads, prostate and breast. VDR activation leads to the maintenance of calcium and phosphorus levels in the blood and to the maintenance of bone content[298].

The catabolic enzyme 24–hydroxylase is responsible for the conversion of both 25(OH)D and 1,25$(OH)_2$D into inactive metabolites and via a multistep pathway to the water soluble calcitroic acid, which undergoes urinary and biliary excretion. 24–hydroxylase is induced by 1,25$(OH)_2$D, which serves as an important feedback mechanism to avoid vitamin D toxicity[298].

Unfortunately, optimal levels of vitamin D have been not universally established. Diversity in measurement methods and reference standards provided by different manufacturers of laboratory kits further complicate the issue of defining cut–points and making judgments in the individual patient. Several prominent investigators reviewed a large number of studies to arrive at an answer to this question. The US Institute of Medicine (IOM) recommendations for vitamin D (IOM 2011)[299], based on a review of the evidence, concluded that:

- serum 25(OH)D < 30 nmol/L is deficient;
- serum 25(OH)D of 30 to 50 nmol/l may be 'inadequate' in some people;
- serum 25(OH)D > 50 nmol/l is 'sufficient' for almost the whole population.

According to these criteria, a high prevalence of vitamin D deficiency or inadequate vitamin D status in Europe has been described. This is especially true for elderly populations, leading to severe consequences in terms of falls, osteoporosis and fractures. The prevalence of vitamin D insufficiency and severe deficiency among postmenopausal patients treated for early breast cancer have also reported to be very high[300]. Moreover, it is now recognized that the target level of 25(OH)D for vitamin D repletion is likely to differ for different tissues[301].

8.2. EFFECTS OF VITAMIN D ON AIA

One possible factor underlying AIA is the high prevalence of vitamin D insufficiency or deficiency in women with breast cancer. Hypovitaminosis D has been suggested as an underlying etiology in individuals with persistent, nonspecific musculoskeletal pain, comparable with the symptoms of osteomalacia[302]. Both RCTs and observational studies have reported promising results that vitamin D may play an important role in these symptoms, suggesting beneficial effects on musculoskeletal discomfort[303–305].

Vitamin D and estrogen metabolism are closely tied; Estrogen increases the activity of 1α–hydroxylase[306] and enhances the expression of *VDR* gene[307]. Hence, the drop in estrogen levels caused by AI might induce a decrease in active vitamin D levels leading to a vitamin D deficient–like arthralgia syndrome. In addition, AI therapy is believed to increase vitamin D requirements: vitamin D is necessary to induce the expression of *CYP3A4*, an essential system for AI detoxification in the liver. Vitamin D, in turn, can activate the promoter region of the aromatase gene in a number of

tissues[308]. Thus, higher concentrations of vitamin D may attenuate local estrogen deficiency[309]. Moreover, vitamin D has non–skeletal effects on a number of tissues including synovium, muscle and cartilage, with putative roles in arthritis[310]. Lastly, it has been hypothesized that decreased vitamin D may also lead to secondary hyperparathyroidism, with the deposition of unmineralized collagen matrix. As a consequence the hydration and expansion of the sub–periosteal tissue would induce secondary pressure on sensory pain fibbers, causing pain[347]. Vitamin D is also known to affect a number of inflammatory pathways associated with the development and persistence of chronic pain. Overall, Vitamin D exerts anatomic, hormonal, neurological and immunological influences on pain manifestation, thereby playing a role in the aetiology and maintenance of chronic pain states[311].

8.3. EFFECTS OF VITAMIN D ON AIBL

One of the most important roles of vitamin D is to maintain skeletal calcium and phosphate balance to allow passive mineralization of unmineralized bone matrix. Serum $1,25(OH)_2D$ does this primarily by stimulating calcium and phosphorous absorption from the gut. In case of vitamin D deficiency, less calcium will be available for bone mineralization and the PTH level will increase, stimulating both the hydroxylation of 25(OH)D to $1,25(OH)_2D$ and bone turnover, in order to restore serum calcium levels. In periods of severe vitamin D deficiency, the mineral deficit leads to osteomalacia (risckets in children), a bone disorder, characterized by decreased mineralization of newly formed osteoid. Vitamin D status is related to BMD, not only in vitamin D deficient subjects, but also in vitamin D insufficient subjects[312–314]. The direct actions of vitamin D on bone are still not well understood, due to

the multitude of effects on the homeostatic mechanisms and to the differences by species, states of differentiation and responsiveness[315,316].

Whether vitamin D deficiency is a predictor for bone fractures is less clear. Furthermore, it is difficult to distinguish the direct effects of vitamin D on bone from its beneficial effects on muscle that result in decreased falls and thereby, fracture risk[317]. Anyhow, guidelines for cancer treatment–induced bone loss include supplementation with calcium and vitamin D[249,251]. Improved vitamin D status using supplementation has been associated with attenuation of AIBL[303,318].

9. GENETIC STUDY OF COMPLEX TRAITS

In the past two decades, many genes that were implicated in simple (Mendelian) diseases have been identified by using genetic linkage approach. Although these methods have been remarkably successful for the identification of high risk genes, they have low statistical power to detect genes with modest effects involved in complex disorders. They are termed "complex" because they are determined by the sum total of multiple genetic and environmental factors. Genetic association studies are a powerful means of identifying the common variants that underlie the complex traits. In the candidate gene approach, pre-specified genes, usually hypothesized to have a role in the disease, are chosen in an attempt to find an association between the gene and the disease. Identified variants in or near those genes that might either cause a change in the protein or in its expression, or be in linkage disequilibrium with functional changes, are genotyped and tested for a possible correlation with the phenotype of interest by statistical methods[319]. These variants use to be single nucleotide polymorphisms (SNPs), variations at a single position in a DNA sequence among individuals.

The discovery rate has been accelerated by the Human Genome Project and by improved technologies for the genome-wide interrogation of variation. A genome-wide association study (GWAS) is an approach that involves systematic DNA screening with markers evenly spaced throughout the whole genome, without regard to their function or context in a specific gene, with the aim of finding genetic variations associated with a particular complex trait. Such studies are particularly useful in finding genetic variations that contribute to common, complex diseases. However, the magnitude of the

effect for a particular polymorphism use to be quite modest. Higher levels of risk may be conferred according to the combined accumulation of many polymorphisms. Consequently, very large case–control studies would be necessary to quantify the risk associated with a particular SNP accurately.

Hormonal therapy with AI is still facing the challenge of interpatient variability in both therapeutic response and intensity of adverse effects. Although AI efficacy among breast cancer patients has been proved, there is significant variability in the frequency of response rate and adverse effects. As most human complex diseases, the outcomes and toxicity of most medications could be governed by, among other factors, genetic variability.

9.1. GENETIC BASIS OF AIA

Previous sections have highlighted the central role of estrogen in many physiological processes and, especially, in the onset and/or intensity of AI side effects. There is ample evidence indicating that determinants of plasma levels of sex steroids are, at least in part, heritable[320]. In fact, common genetic variants have been related in some extent to the effectiveness of AI treatment, particularly those in *CYP19A1* gene[321–323]. All the foregoing indicates that these and/or other variants may also be involved in the onset and/or intensity of AI secondary effects. Thus, for example, a tetranucleotide repeat polymorphism (TTTA)n, in *CYP19A1* gene, previously associated with circulating estradiol and estrone levels, was already related to the occurrence of AIA[324]. Park et el. also described an haplotype of *CYP19A1* to be associated with bone/joint paint due to the AI–therapy[325]. Moreover, another study found that an intronic variant of *ESR1*, which encodes receptor ERα, is associated with increased risk of AI discontinuation

due to musculoskeletal toxicity[326]. On the other hand, a GWAS study in 2010 also found that SNPs in the T-cell leukaemia 1A gene (*TCL1A*), which, in turn, was related to the IL-17, were associated with musculoskeletal side effects[327].

9.2. GENETIC BASIS OF AIBL

Studies in twins and families indicate that genetic factors play an important role in the regulation of BMD, estimating its heritability between 50% and 85%[328,329]. Osteoporosis is a polygenic disorder, determined by the effects of several genes, each with relatively modest contribution to bone mass and other determinants of fracture risk. Candidate gene studies have revealed several genes involved in the pathogenesis of the disease including *COL1A*, *LRP5, CYP19A1, SOST, TGFβ1, ESR*, *AR*, *VDR*, *IL-6*, calcitonin receptor, *OPG*, *RANK* and *RANK-L*[330]. GWAS have also identified several loci influencing BMD variation[331–336]. A genome-wide meta-analysis in 2012 identified 56 loci (32 novel) associated with BMD, of which 14 also yielded significant association with fracture risk[337]. Several of these factors cluster within the RANK-RANKL-OPG, mesenchymal-stem-cell differentiation, endochondral ossification and the WNT signalling pathways. However, loci containing genes not known to play a role in bone biology were also discovered. Although these findings helped explain a large amount of genetic architecture underlying BMD variation and fracture susceptibility, the understanding about genetic factors contributing to osteoporosis response is still limited.

As regards the specific case of AIBL, whether the already identified variants, involved in BMD determination or AI response, contribute to the

pathogenesis is still unknown. Population analysis involving larger patient cohorts and examining all functional gene variants simultaneously is required to validate the existing findings and to elucidate the mechanisms underlying the variability of the secondary effects of AI. Tests that are able to predict treatment response might allow us to identify upfront patients who will not tolerate AI, providing valuable information for treatment planning.

Chapter 2

RESULTS

La ciencia está maravillosamente preparada para
responder a la pregunta ¿Cómo?,
pero se vuelve terriblemente confundida cuando se
enfrenta a la pregunta ¿Por qué?

Erwin Chargaff

GENETIC DETERMINANTS OF AROMATASE INHIBITOR-RELATED ARTHRALGIA: THE B-ABLE COHORT STUDY

Garcia-Giralt N, Rodríguez-Sanz M, Prieto-Alhambra D, Servitja S, Torres-Del Pliego E, Balcells S, et al. Genetic determinants of aromatase inhibitor-related arthralgia: the B-ABLE cohort study. Breast Cancer Res Treat. 2013 Jul 19;140(2):385–95. DOI: 10.1007/s10549-013-2638-3

AI-RELATED BMD VARIATION IN ACTUAL PRACTICE CONDITIONS: A PROSPECTIVE COHORT STUDY

Rodríguez-Sanz M, Prieto-Alhambra D, Servitja S, Garcia-Giralt N, Garrigos L, Rodriguez-Morera J, et al. AI-related BMD variation in actual practice conditions: A prospective cohort study. Endocr Relat Cancer. 2016 Apr;23(4):303–12. DOI: 10.1530/ERC-16-0025

CYP11A1 EXPRESSION IN BONE IS ASSOCIATED WITH AROMATASE INHIBITOR-RELATED BONE LOSS

Rodríguez-Sanz M, García-Giralt N, Prieto-Alhambra D, Servitja S, Balcells S, Pecorelli R, et al. CYP11A1 expression in bone is associated with aromatase inhibitor-related bone loss. J Mol Endocrinol. 2015 Aug 24;55(1):69–79. DOI: 10.1530/JME-15-0079

EVOLUCIÓN DE LA DMO DURANTE EL TRATAMIENTO CON INHIBIDORES DE AROMATASA Y SU RELACIÓN CON EL GEN CYP11A1: ESTUDIO PROSPECTIVO DE LA COHORTE B-ABLE

Rodríguez-Sanz M, Prieto-Alhambra D, Servitja S, García-Giralt N, Garrigos L, Albanell J, et al. Evolución de la DMO durante el tratamiento con inhibidores de aromatasa y su relación con el gen CYP11A1: estudio prospectivo de la cohorte B-ABLE. Rev Osteoporos y Metab Miner. 2015 Dec;7(4):98–105. DOI: 10.4321/S1889-836X2015000400004

TBS AND BMD AT THE END OF AI-THERAPY: A PROSPECTIVE STUDY OF THE B-ABLE COHORT

María R-S, Marta P-M, Sonia S, Natalia G-G, Tamara M, Ignasi T, et al. TBS and BMD at the end of AI-therapy: A prospective study of the B-ABLE cohort. Bone. 2016 Nov;92:1–8. DOI: 10.1016/j.bone.2016.08.008

Chapter 3

DISCUSSION

Este hombre, por una parte, cree que sabe algo,
mientras que no sabe [nada].
Por otra parte, yo, que igualmente no sé [nada],
tampoco creo[saber algo].

Apología de Sócrates-Platón-

AI are currently indicated as first-line adjuvant endocrine therapy in the treatment of early-stage breast cancer in postmenopausal women with ER-positive tumours. Since 2001, the efficacy of third-generation AI has been well established and large clinical trials have shown these agents to be superior to tamoxifen in terms of disease-free survival, incidence of contralateral breast cancer and time to recurrence[147]. The efficacy of AI has led to a considerable increase in the frequency of their use. Consequently, the complications arising from AI therapy in this patient population have long-term effects and greatly impact patient quality of life.

Most of the epidemiological data concerning AI secondary effects originates from RCTs, which are the most rigorous strategy for determining a cause-effect relation between an intervention and an outcome. RCTs assess the effect of the intervention by comparing it to a control condition, standard treatment or placebo. Randomization of participants to the test and control arms and concealment of their allocation ensures that allocation bias and confounding of unknown variables are minimized. Although RCTs are powerful tools for causal inference, their use is not discharged from constraints. The strict and controlled conditions in which they are conducted, often confines their applicability to ideal conditions and this limits their ability to portray what happens in the *real-life* population[338].

Therefore, one of the main purposes of the present work was to describe the prospective evolution of musculoskeletal adverse effects of AI treatment in the *real-life* usual care. In this context, patient characteristics, as well as therapy adherence, may differ from those observed in RCTs. Observational studies mimic everyday clinical practice, providing higher external validity. For this purpose, the B-ABLE cohort, a prospective, observational, clinical

cohort study was available. All the postmenopausal women diagnosed with early breast cancer and candidates for AI treatment attending the outpatient Breast Cancer Unit, Hospital del Mar (Barcelona, Spain), were consecutively invited to participate in this study and recruited after informed consent. Information on a large number of clinical variables was recorded at the time of enrolment, including the intensity of self-reported joint pain and BMD at baseline and repeated at each follow-up visit.

JOINT PAIN IN THE B-ABLE COHORT

Pain is defined as"an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described by the patient in terms of such damage". Pain processes do not begin with the stimulation of receptors. Rather, injury or disease produces neural signals perceived in the conscious brain, which participate in the selection, abstraction and synthesis of information from the total sensory input. Thus, pain is essentially a subjective perceptual experience influenced by a complex interaction of behavioral, environmental, biological and social factors[339].

In the B-ABLE cohort, pain is measured by the visual analogic scale (VAS). The VAS consists of a 10-cm line with the two endpoints labelled as "no pain" and "worst pain ever" (or similar verbal descriptors) (**Fig. 17**). The question associated to the VAS reads as follows: "Please, score the intensity of the pain you feel in your peripheral joints (knee, wrist, fingers/toes, elbow, shoulder, etc.), excluding spine/back pain and pain at the operated area" (translated from Catalan/Spanish by the authors). Patients are required to place a mark on the 10-cm line at a point that corresponds to the level of pain intensity. The distance in cm from the low end of the VAS to the

patient's mark is used as a numerical index of the severity of pain.

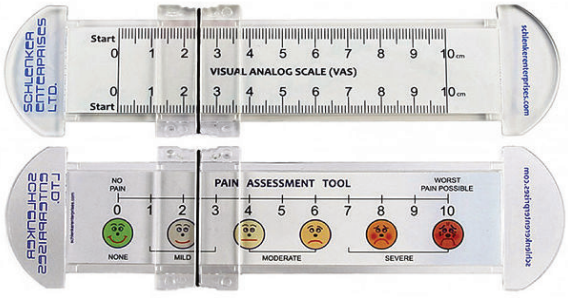

Figure 17. VAS pain scale ruler. The scale is most commonly anchored by "no pain" (score of 0) and "worst imaginable pain" (score of 10). Extracted from: http://www.custompromotionalrulers.com/visual-analog-scale-vas-rulers/vas-pain-scale-rulers-0-10-cm-w/slider/

The major disadvantage of VAS is the assumption that pain is an unidimensional experience that can be measured with a single-item scale of intensity[339]. Notwithstanding, patient reported toxicity more comprehensively capture the subjective side effects of therapies (that is, pain) on daily experience and has higher concordance with health-related quality of life than clinician ascertained toxicity; therefore, it is more appropriate for the investigation of AIA[340]. Additionally, VAS's ratio scale properties make it appropriate to speak meaningfully about percentage differences between VAS measurements obtained at multiple points in time. Other advantages of the VAS include its ease and brevity of administration and scoring, minimal intrusiveness and conceptual simplicity.

VAS is sensitive to pharmacological and nonpharmacological procedures that alter the experience of pain and correlate highly with pain measured on verbal and numeric rating scales[341]. Unfortunately, in our study, details of

non–steroidal anti–inflammatory drugs and other pharmaceutical interventions usually indicated for pain amelioration were not collected. This is an important limitation to consider, since it may be biasing the results obtained for AIA.

A previous study in the B–ABLE cohort, found that the mean joint pain increased from VAS score of 3 at baseline to 4.5 at 3 months follow–up[305]. In our analysis, about 50% of women reported worsening pain both at 3 and 12 months of AI–treatment[342]. Moreover, approximately 5% of women in the B–ABLE cohort discontinued treatment due to severe AIA.

Some studies have demonstrated by sonographic, electrophysiologic and magnetic resonance imaging measurements that patients with AI–related arthralgia often have changes in their affected joints and tendons[176,177]. We also aimed to explore this possibility in the B–ABLE cohort. Thus, dynamic ultrasonographic exploration in cross–sectional and longitudinal areas of 10 joints of the hands (carpus, 10 metacarpophalangeal and 10 proximal interphalangeal joints) through ESAOTE Mylab 60 equipment (multifrequency probe of 7–13 Hz equipped with Doppler) was conducted. The exploration method and the diagnostic criteria for synovial effusion, synovial hypertrophy and tenosynovitis are those described and recommended by the working group OMERACT–7[285]. The presence of synovial hypertrophy, effusion and the intra–articular power–Doppler signal are being evaluated by a semi–quantitative scale from 0 to 3. In addition, carpal tendons with synovial sheath and finger flexors are included. A global inflammation index resulting from the sum of the scores in both hands has been defined. So far, no significant morphologic changes in the affected joints and tendons have been found.

A process of central sensitization might also be considered. Persistent pain alters the nervous system, lowering the threshold for pain generation and increasing the duration, amplitude and spatial distribution of pain.

BMD EVOLUTION IN THE B-ABLE COHORT

The gold-standard for estimation of BMD is the DXA technique because of its reproducibility, large normative data, non-invasive nature, little time requirement for procedure and minimal radiation exposure. However, it is essential to be aware of some pitfalls in the interpretation of BMD report. The precision achievable is dependent both on the machine and the subject being measured, and it is limited by statistical error of the signal, the accuracy of the detection of the bone edge and the reproducibility of subject positioning. All the DXA scans of the B-ABLE patients have been performed on the same machine and by the same technician, minimizing thus the potential variability associated with some of these factors. However, while assessing BMD, scan artefacts must be screened since they may lead to overestimation of the BMD. Technical artefacts include metallic objects like surgical clips, navel rings, barium sulphate, metal from zipper, coin, clip, or other objects. A number of diseases may also interfere with the accuracy of the BMD measurement. Thus, degenerative disc disease with osteophytes (radiographic markers of spinal degeneration representing enlargements of the normal bone structure), spondylosis, osteoarthritis, scoliosis, aortic calcification and vertebral fractures can also be present, particularly affecting patients aged 60 years or more[343] (**Fig. 18**).

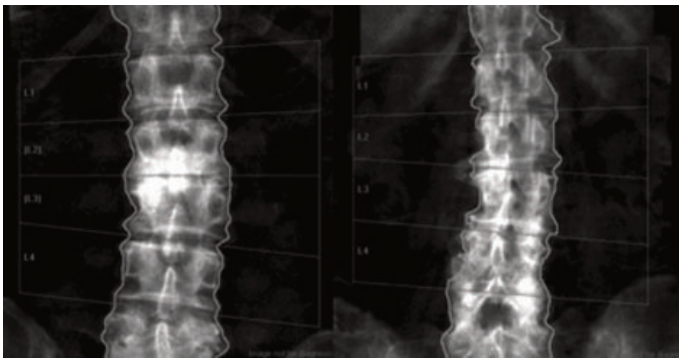

Figure 18. Example of spinal degenerative changes in a DXA scan. Extracted from Buehring et al. (Journal of Caquexia, Sarcopenia and Muscle, 2013)[344].

The effect of osteophytes can be dramatic: the magnitude of increase in BMD due to osteophytes can vary from 9.5% at L4 to 13.9% at L1[345]. Thus, it has been reported that the effect of osteophytes on BMD is sufficient enough to cause 25% of women with osteopenia and 10% of women with osteoporosis to be misdiagnosed[343]. Therefore, material correction by removal of artefacts, or exclusion of the affected vertebra should be a mandatory requirement. In patients with extensive degenerative disease, the spine scan may be of little diagnostic value. Inspection of scan images is particularly important when interpreting follow-up scans and visual comparison should always be made with previous studies. Hip scans also require careful scrutiny as there is a wide range of anatomical variations, some of which cause difficulties in correctly positioning the hip. However, structural changes and artefacts interfering with DXA at proximal femur are less often as compared to spine[346]. In our study, those follow-up scans results showing unexpectedly large rates of change were reviewed to exclude the mentioned factors as a cause. Our results indicated that LS BMD is substantially influenced by degenerative factors: when excluding from the

analysis those patients with scan artefacts, LS BMD reductions were more pronounced (**Fig. 19 A**). All this suggests that the spine AIBL decline in the whole cohort is partially obscured by degenerative changes.

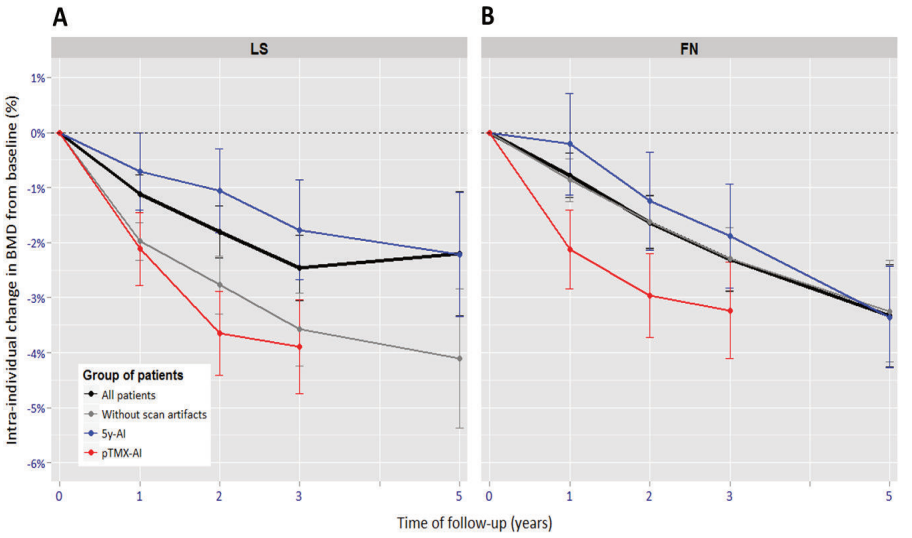

Figure 19. Summary of BMD evolution in the B-ABLE cohort patients without BP treatment. Results are presented as intra-individual percent change on LS and femoral neck (FN) BMD from baseline to the end of AI therapy.

Nearly all data available indicate that AI are superior to tamoxifen[148]. However, the decline of plasma estrogen induced by AI is definitely associated with the reduction of BMD[245–248] and increased risk of bone fracture[154]. The observed BMD decreases throughout the entire AI therapy in B-ABLE patients were, generally, milder than those reported in previous RCTs[246,247,276,347,348]. Indeed, in some of these trials, women experienced mean FN BMD decreases almost twice as much as B-ABLE cohort patients.

There are a number of factors that can contribute to this phenomenon. First,

some baseline patient characteristics can determine differences in BMD variation throughout the follow-up. Thus, for example, differences in lifestyle and dietary factors between the study cohorts should be kept in mind. Moreover, the lower baseline BMD values in the B-ABLE cohort, as compared with those reported in most of the mentioned studies, can lead to a regression to the mean bias. That is, those patients starting with low levels of BMD are less prone to experience greater BMD decreases. Accordingly, significant association has been observed between baseline BMD and BMD changes in other studies[349].

Second, the strict monitoring of BMD as well as calcium and vitamin D supplements may also contribute to the less pronounced BMD reductions. High vitamin D deficiency rates at baseline have been described in the B-ABLE cohort (~90% of patients). However, after 3 months of vitamin D supplementation >70% of women were replete (**Fig. 20**).

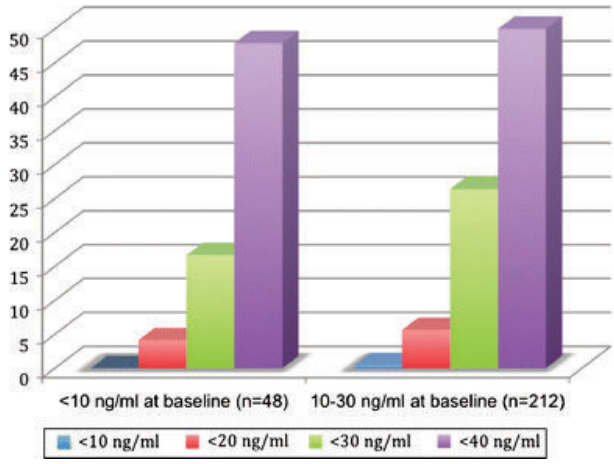

Figure 20. Vitamin D levels in the B-ABLE cohort. Percentage of women with vitamin D serum levels below different thresholds (10, 20, 30 and 40 ng/ml) after 3 months of supplementation with $VitD_3$ daily 800 IU + 16,000 IU every 2 weeks, among patients

who were vitamin D insufficient and deficient at baseline. Extracted from Prieto-Alhambra et al. (Breast Cancer Research and Treatment, 2011)[305].

Both higher vitamin D levels and vitamin D increases at 3 months have already been associated to lower BMD loss in B-ABLE patients[350], supporting the hypothesis that vitamin D repletion can play a protective role against AIBL. The combined benefit of bone loss attenuation and decreased AI-associated arthralgia strengthens the case for 40 ng/ml level as the optimal therapeutic target. Thus, an individualised vitamin D supplementation regimen depending on patient characteristics and antecedents should be considered.

Third, the steroidal structure and putative androgenic activity of exemestane might have different effects on bone compared with the nonsteroidal AI[351]. Some studies have even noticed BMD increases associated to this agent, although not statistically significant[352]. In the B-ABLE cohort, nearly 30% of women receive exemestane. This considerable proportion might also account to the lower BMD decreases in the B-ABLE cohort.

Finally, inherent differences between the study design of B-ABLE (observational study) and RCTs can also lead to differences in the mentioned outcome. Accordingly, another observational study on the impact of AI on bone fractures found similar BMD losses at 3 years of follow-up than those reported in our work[353].

Previous tamoxifen treatment also modifies the bone loss rate. Thus at the end of AI-treatment, those patients who had received previous tamoxifen (pTMX-AI) experienced equal or even higher BMD reductions in half the time that patients receiving AI only (5y-AI) (**Fig. 19**).

It is not easy to identify the exact cause(s) of this aspect. Several studies have suggested that the effects of tamoxifen on bone resemble those of estrogen, preserving bone even in patients with breast cancer[354–356]. Unfortunately, this protective effect could become a disadvantage, leading to a rebound effect: the early increase in BMD with tamoxifen may be followed by a steep decrease when switching to an AI. However, differences in some other characteristics (for example, tamoxifen is given to perimenopausal women) cannot be ruled out as a possible causes of the difference in BMD loss rates between both groups of our study. In spite of this, the more accelerated BMD loss in pTMX–AI patients does not seem to have consequences in absolute terms. That is, BMD values at the end of treatment in pTMX–AI and 5y–AI groups were not statistically different.

EFFECTS OF BP ON BMD EVOLUTION

In the B–ABLE cohort, women with osteoporosis or osteopenia plus 1 major risk factor or prevalent fragility fractures were allocated to oral BP treatment (BP–treated patients). This group of patients experienced significant increases in both LS and FN BMD (**Fig. 21**).

As B–ABLE is not a RCT, we cannot assume that BP are responsible for the observed BMD increases: a group of patients with osteoporosis at baseline but without BP treatment would be strictly necessary to draw any conclusions about treatment efficacy. In our study, pre–existing baseline differences between BP–treated and BP–untreated groups could influence the study results: both groups differ naturally, at least, in BMD values at baseline. Thus, for example, those patients with lower BMD at baseline would be expected to experience lower BMD rates, merely for a regression to the

mean effect. However, the clear BMD increase observed exclusively in BP–treated patients suggests an effect attributable to these agents. Neither can BMD increases be ascribed to vitamin D and calcium supplementation, since the BP–untreated group is also receiving the same doses of such supplements. In this sense, reliable evidence has been published about the effects of BP on BMD. In several randomized trials, BP significantly increased BMD in postmenopausal women with early breast cancer receiving AI[248,272]. Therefore, we consider that BP therapy is the most likely cause of the observed mean BMD increase BP–treated patients.

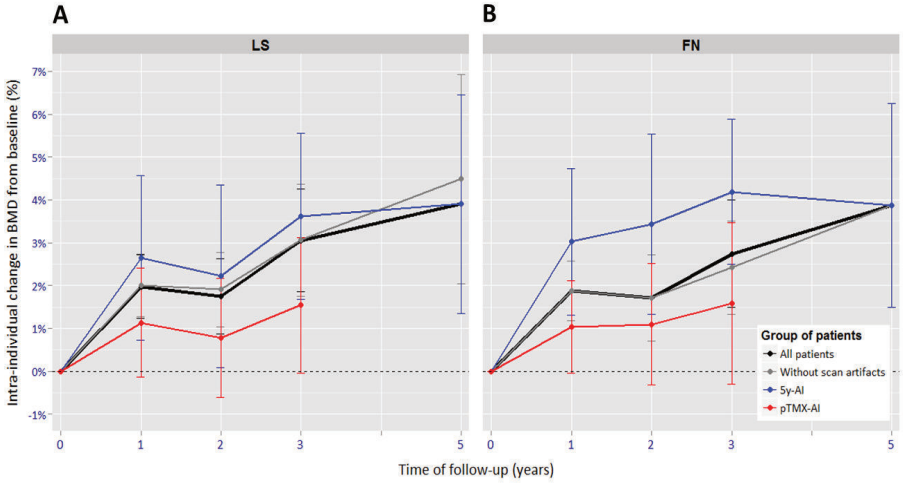

Figure 21. Summary of BMD evolution in the B-ABLE cohort patients with BP treatment. Results are presented as intraindividual percent change on LS and FN BMD from baseline to the end of AI therapy.

The greatest BMD increases took place during the first year of the study. As for the subsequent time–points, BMD increases did not yield statistical significance. Nevertheless, this effect may be caused by the substantial decrease in the sample size during the follow–up and therefore the analyses

should be replicated when higher sample size is reached.

BP therapy seems to induce greater BMD increases in 5y–AI group. In fact, pTMX–AI patients did not achieve significant BMD increases with BP therapy. The rebound effect of tamoxifen could also be contributing to this phenomenon.

It is noteworthy that even though BP–treated patients experienced significant BMD increases, their absolute BMD values never reached those of non–BP–treated patients. Thus, by the end of treatment, average BMD values for BP–treated group still remained below those of BP–non–treated patients. In fact, although non–BP–treated patients experienced substantial BMD decreases, only a few women became osteoporotic during the follow–up. If we strictly adhere to preventing bone loss properties of BP one may state that they should only be prescribed to osteoporotic patients. Some other studies indicate that AIBL is relatively predictable and, thus, for women with normal BMD before starting AI therapy, the risk of developing osteoporosis over 3 to 5 years on AI is very low; Lifestyle advice, reassurance, and very limited, if any, follow–up measurements of BMD are all that is required[247]. We nevertheless have to bear in mind that BP could, in addition, provide oncological benefit. Thus, a recent meta–analysis has demonstrated that adjuvant BP reduce the rate of breast cancer recurrence in the bone and improve breast cancer survival in postmenopausal women[357]. Overall, adjuvant BP may be considered in a broader range of postmenopausal women.

BONE MICROARCHITECTURE: A POSSIBLE ROLE FOR TBS.

The measure of TBS has been proposed as a clinical tool capable of assessing trabecular bone microarchitecture. In an attempt to create an analogy with the three BMD categories, a working group of TBS users from different countries established cut-off points creating the following range for TBS values in postmenopausal women: TBS $\geqslant$1.350 is considered to be normal; TBS between 1.200 and 1.350 is considered to be consistent with partially degraded microarchitecture; and TBS $\leqslant$1.200 defines degraded microarchitecture[358]. At baseline, most patients in the B-ABLE cohort had a partially degraded microarchitecture and particularly, the degraded microstructure category prevailed in patients allocated to BP treatment.

In our study, TBS experienced significant mean decreases in the non-BP-treated patients at the end of treatment[359]. Images with degenerative changes were not excluded in this particular observational assessment. In this sense, some studies have shown that degenerative changes have little effect on TBS. However, taken into account the effects of osteophytes on BMD, it is probably that TBS reductions are less pronounced than the true BMD declines.

Tamoxifen has also demonstrated to induce a compensation and/or stabilization of bone texture parameters[291]. Similarly to BMD, TBS also decreased more sharply in pTMX-AI group[359]. The same rebound effect described for BMD may also be the underlying cause.

In contrast to BMD, TBS did not increase in BP-treated patients and remained unchanged from baseline[359]. As with BMD increases, we cannot grant that the stabilization of TBS is caused by BP. However, similar observations were made in RCTs evaluating the effects of BP on TBS[360]. Overall, TBS is sensitive

to changes in estrogen status and remains unchanged when BP therapy is added. This is not surprising, because one would expect a greater improvement on BMD with antiresorptive therapy, resulting from increased mineralization, than in trabecular microstructure.

TBS stems from the need to capture those fragility fractures occurring in the osteopenic or even the normal range of BMD. In our study, changes in TBS and BMD were only weakly correlated, supporting the contention that the two measures reflect different aspects of bone status[359]. However, it remains to be stablished whether TBS variation exert a clinically relevant effect on fracture risk. Moreover, there is some data suggesting that AI substantially affect cortical bone compared with trabecular bone[361]. The incidence of hip or femoral fracture among postmenopausal women undergoing breast cancer therapy seems to be higher than the incidence of vertebral fractures. Furthermore, hip fractures occurred much earlier in women with breast cancer than in healthy women[362]. Until now, TBS has only been implemented for spine, so it may overlook some a portion of the risk of hip fracture.

HIGH VARIABILITY IN THE STUDIED OUTCOMES

In the previous sections, adverse effects of AI in the B-ABLE cohort have been described in a comprehensive manner. There is no question, however, that one of the most remarkable findings in this work is the great variability in the magnitude of the studied outcomes.

Although one can state that median VAS for joint pain increased from baseline to 3 months of follow-up in the B-ABLE cohort, it is noteworthy

that only 50% of women with no joint pain at baseline reported incident joint pain at 3 months. From those with prevalent join paint at baseline, 50% reported had a worse VAS at 3 months but 25% reported no change and approximately 25% even got better[305,342].

As regards BMD variation during AI treatment, these differences became evident when analysing patient distribution by BMD change categories. While it is true that most of BP–non–treated patients undergone clinically significant reductions in BMD, just over a third of women in this group hadn't yet experienced any change in LS and/or FN BMD by the second year of AI[363]. Some previous studies have also reported such BMD variability[250].

Similar variation was detected in TBS. At the end of AI–therapy, approximately 25% of non–BP–treated patients decreased by one TBS category, while the remaining individuals persisted in their baseline category. Even more variability was detected in the BP–treated group, in which 14% of women raised from TBS category[359].

Several sources of technical and/or biological variability may underlie these observations.

- Scan artefacts can cause falsely BMD measurements[345]. Thus for example, some BMD increases >3% observed in our study could be explained by these anomalies. However, BMD variability remained even after the exclusion from the analysis of those patients with scan artefacts[364]. Moreover, FN BMD variation, which is not so susceptible to the presence of osteophytes, also presents high inter–patient variability. Consequently, other relevant factors must be considered[363,364].

- Coefficient of variation (CV) of DXA: CV is the most commonly presented measure for BMD scans reproducibility and it depends on quality assurance factors, including quality control tests, performance of the machine as well as the experience of the operator. The small unexpected changes in BMD observed in some patients could be attributed to the CV, but those changes higher than 3% can hardly be explained by this phenomenon.

- Despite a cumulative loading of 168,000 IU of vitamin D supplements, a clinically significant proportion of women failed to attain adequate vitamin D status within 3 months[305]. As previously mentioned, an association between vitamin D serum levels and AIBL has been described: those patients with improved vitamin D status using supplementation showed attenuation of bone loss[350]. In this sense, vitamin D supplementation may also help to explain a portion of the observed BMD increases in those patients who are not receiving BP. Furthermore, the increase in joint pain was significantly attenuated in those that reached high vitamin D concentrations, with a lower risk of incident arthralgia[305]. Thus, the interindividual differences in vitamin D improvement/status may be influencing the variability of both AIBL and AIA.

- While studies have found that adjuvant hormonal therapy for hormone–sensitive breast cancer dramatically reduces recurrence and mortality, adherence to medications is suboptimal[185–189]. Non–adherence may also constitute a plausible justification for an important part of the interindividual variability in the treatment–associated secondary outcomes. In the B–ABLE cohort, self–reported adherence to AI, BP and vitamin D supplements is assessed by physician questionnaire at each

time-point. Self-report is a widely accepted and applied method to assess medication adherence, however, this may be less reliable to fully reflect true adherence. This, in fact, is one of the limitations of our study. Non-adherence can either affect AI, BP as well as calcium and vitamin D supplements. Therefore, a "combination" of treatment efficacies can be derived, leading to a large variability in the secondary outcomes. Thus, for example, some authors have already associated BMD loss in BP-treated patients with poor adherence to BP treatment[353].

- Emotional state, individual's evaluation of his/her own health condition, social support, health beliefs, education level, psychological problems as well as socioeconomic status can also influence pain feeling[365]. These variables are, in turn, closely linked to treatment compliance[299]. Thus, psychotherapy including relaxation training, biofeedback, visual imagery, distraction methods and psychiatric intervention could effectively alleviate pain-related emotional stress and depression[366].

- Besides the reported clinical factors, genetic background may also be of importance to interindividual variability. Hence, some genetic variants in *CYP19A1*[324,325], *ESR1*[326] and *TCL1A*[327] had been associated so far with AIA. Accordingly, in our study, a number of SNPs in *CYP17A1*, *CYP27A1* and *VDR* genes were associated with both joint pain intensity and worsening during AI-therapy. Moreover, one SNP in *CYP27A1* was also related to AI-therapy discontinuation due to severe arthralgia[342]. Subsequent to our study, Lintermans et al.[367] tested the validation of SNPs in selected genes, based on literature review, with AI-related musculoskeletal symptoms. They only found one SNP in *OPG* gene yielding significant association. No correlation between any other tested SNPs, including

those in *CYP17A1* identified in our study, was found. In accordance, another study also found *OPG* and *RANKL*[368] to be related to AI musculoskeletal side effects. Differences in definitions of outcomes and patient cohorts used are probably responsible for these inconsistencies.

We have also described an association between SNPs in *CYP11A1* and bone loss[364]. Other studies found additional polymorphisms to be associated with bone loss among patients taking AI, during the course of our work. Thus, Napoli et al.[369] reported one SNP in *CYP19A1* gene to be associated with AIBL. Another study in 2015 found that SNPs in *ESR1*, *ESR2* and *CYP19A1* were associated with decreased bone density in letrozole and exemestane–treated patients[348]. Moreover, a GWAS in 2014[370] identified SNPs in or near *CTSZ–SLMO2–ATP5E*, *TRAM2–TMEM14A*, and *MAP4K4* genes that were associated with risk for bone fracture in AI–treated women. Interestingly, these genes all displayed estradiol (E2)–dependent induction. The discovery of such large number of associations of SNPs in genes involved in estrogen synthesis pathway with AI secondary effects (and/or effectivity) suggests, once again, that the estrogen environment is essential for the tolerability of these drugs. In our work, SNPs in *CYP17A1* and *CYP11A1* have been associated with AI side effects. As previously mentioned, steroidogenesis regulation is determined by *CYP11A1* (encoding P450scc protein) gene expression and other downstream enzymes, especially P450c17 (encoded by *CYP17A1*). These genes, therefore, have a key role in the local sex steroid hormone levels, probably influencing also some AI secondary outcomes such as AIBL and AIA.

INTERINDIVIDUAL DIFFERENCES IN SEX-STEROID LEVELS: A POSSIBLE UNDERLYING MECHANISM FOR VARIATION IN AI SECONDARY EFFECTS.

Despite the difficulties in measuring in vivo estrogen concentrations, it is overall accepted that AI reduce whole-body aromatization by 90.6% – 98.9%. However, subtle differences in the degree of hormone suppression among the 3 third-generation AI have been described[145]. More interesting, though, is that some studies have reported unexpectedly high plasma estrogen levels in AI-treated patients. Thus Kallak et al.[371] evaluated aromatase index in long-term AI treatment, showing that the pattern of estrogen levels is more unpredictable than the described in previous RCTs. In addition, a wide interindividual range of concentrations of circulating estradiol and estrone were reported.

We personally agree with the findings indicating such a high interindividual variability in estrogen levels during AI therapy. In fact, we hypothesize that this is one of the mechanisms underlying the wide range of intensities associated to these medications.

Poor drug potency and alternative sources of estrogenic hormones are only likely to be the cause of the interindividual variability in estrogen levels during AI therapy in occasional cases. Non-adherence and drug-interactions also constitute a plausible justification for an important part of this interindividual variation[372]. Moreover, a positive relationship has been found between estrogen levels and BMI during treatment with AI[373]. Adipose tissue is the main source of estrogen biosynthesis in postmenopausal women, so that incomplete estrogen depletion in women with high BMI can occur, even leading to reduced efficacy of AI in obese women[408,409]. In spite of this, Kallak

et al. stated that the observed interindividual differences among AI-treated patients in their study could not be attributable to individual differences in BMI nor other variables as time since menopause or self-reported adherence[371]. Therefore, other factors may be contributing to this phenomenon.

We hypothesize that genetic variants are responsible, at least in part, of the interindividual variability in host sex-steroid and vitamin D environment during AI-therapy. This, in turn, translates into the interindividual variability in the secondary effects (and effectivity) of AI (**Fig. 22**).

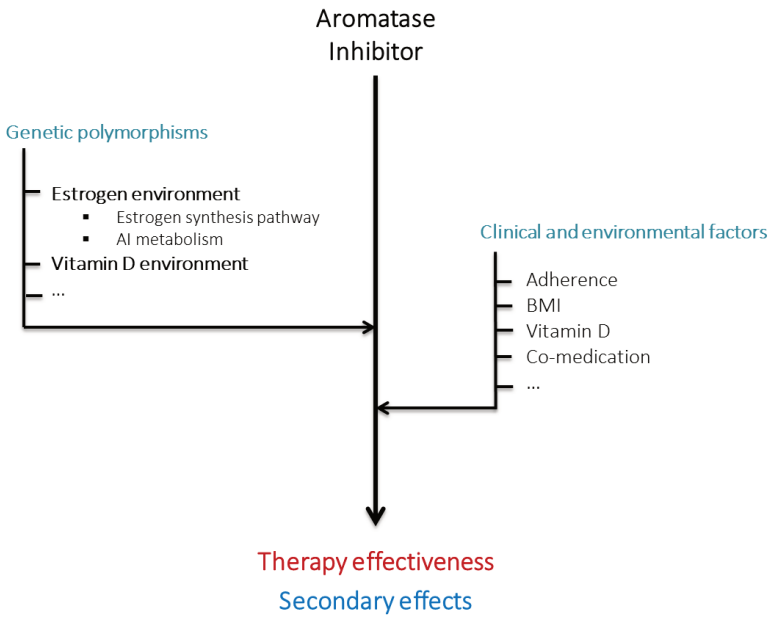

Figure 22. Scheme of the hypothetic mechanisms underlying the interindividual variability in AI effectivity and in their associated side effects.

Some findings would underpin our hypotheses; First, there is a wide range in

the response rates to AI: percentages between 35 and 70% in neoadjuvant studies have been reported and benefits may be lower in advanced disease[374]. In that connection, resistance to AI has already been associated with SNPs in *CYP19A1*[375]. In fact, it has been already demonstrated that high/raised levels of aromatase may prevent effective blockade by inhibitors. Additionally, the appearance of AI unwanted effects has been demonstrated to be a predictor of endocrine treatment response[193,376]. Therefore, one could infer that genetic variation determining therapy effectiveness may also influence the onset and/or intensity of adverse effects.

Second, the importance of the low residual estrogen in breast cancer patients has already been demonstrated. Thus, the ATAC trial detected an inverse correlation between baseline estradiol levels and BMD changes: lower baseline estradiol is a predictive not only of lower baseline BMD but also of greater BMD losses during AI therapy. Remarkably, no other variables retained significance after these covariates were included in the model[349]. Before the widespread use of AI in breast cancer patients, the importance of the low residual estrogen levels in postmenopausal women was already demonstrated. Thus, for example, the lower levels of serum estradiol are associated with the higher rates of bone loss and the greater risk of fractures in late postmenopausal women[377]. The mechanism by which such low estrogen concentration becomes so important remains unknown. However, some clinical observations suggest that long–term deprivation of estradiol may cause adaptive hypersensitivity to low levels of estradiol[357]. In fact, there is some evidence that women starting AI who develop joint manifestations can experience improvement over time[165] since tissues may

adjust to the low estrogen concentrations[378].

In short, those women with a genetic background "favouring" a successful inhibition of estrogen synthesis by AI would show high intensity–side effects, indicating, in turn, higher likelihood of responding to endocrine treatment. On the contrary, a lack of estradiol response would not trigger symptoms but, unfortunately, breast cancer recurrence prevention would be lower. If true, new predictive models for hormone treatment response and side effects intensity could be achieved by combining genetic tests with other clinical predictive factors. In this sense, SNPs *CYP11A1, CYP27B1, VDR* and *CYP17A1* are likely good candidates for this purpose.

THE ROLE OF LOCAL ESTROGEN SYNTHESIS

Even if circulating estrogen levels are often used to evaluate AI treatment efficacy, its importance has been questioned. No correlation between local and plasmatic estrogen levels has been described. This is supposedly due to differential uptake from the circulation and/or local estrogen production. The ability to convert cholesterol into pregnenolone via P450scc constitutes the essential prerequisite for a tissue to be steroidogenic. The presence and biochemical activity of P450scc and P450c17, documented in some tissues as human skin and brain, changes the paradigm of the steroidogenic capacity confined so far to the adrenal gland, ovaries, and testis. Bone has long been regarded as just one of those compartments with capacity to synthesize estrogens from serum androgen precursors. However, in our study, the expression of P450scc and P450c17 at both mRNA and protein level has been also detected in bone cells[364]. This finding evidences that bone possess all the enzymatic machinery for *de novo* estrogen synthesis, opening the possibility

for this tissue to be considered as a steroidogenic compartment. However, further work is mandatory in order to demonstrate the enzyme functionality in this compartment.

The corroboration of bone as steroidogenic tissue would entail a growing importance of local estrogen synthesis. The challenge will come in determining the functional significance of this pathway, since its contribution to local estrogen levels may have important therapeutic implications. The importance of this aspect lies in the fact that the low circulating levels of estrogens which are observed in postmenopausal women have no bearing on the concentrations of estrogen reached in extragonadal site. The significance of this paradigm shift cannot be underestimated, namely that the estrogen which is responsible for breast cancer development and for the maintenance of bone mineralization and cognitive function is not circulating estrogen but rather that which is produced locally at these specific sites within the breast, bone and brain.

This raises the possibility of using pharmacologic organ or tissue–specific estrogen deprivation as an improved means of targeted therapy. For example, selective local induction of this pathway in bone/joint can be of therapeutic benefit in a context of estrogen reduction while suppressing breast estrogen levels.

P450SCC IN BONE: FURTHER UNKNOWN FUNCTIONS?

P450scc have two known isoforms, the full length 60–kDa (521 aa) protein and a unique shorter isoform known as isoform b of 42–kDa (363 aa), missing aa from 1 to 158 from the canonical sequence (**Fig. 23**). This shorter

isoform is regulated by alternative splicing and utilization of an internal translational start codon. Unlike the full-length protein, isoform b is not concentrated into mitochondria.

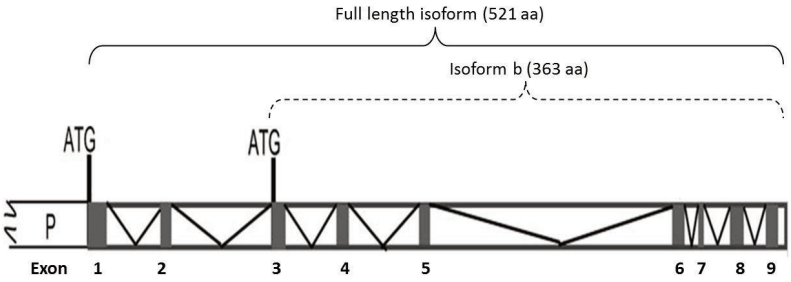

Figure 23. Full-length and short isoform transcripts of *CYP11A1* gene. Adapted from: Teplyuk et al. (Molecular Endocrinology, 2009)[379].

In our analysis, both isoforms of P450scc were detected in human primary osteoblasts and fresh bone tissue[364]. In fact, Teplyuk et al. had already detected the expression of the short isoform in mouse and human osteoblastic cell lines[379]. Although its function remains unknown, they found that knockdown of this isoform b increased osteoblast proliferation, suggesting that it contributes to Runx2-mediated attenuation of cell growth. There are still outstanding tasks in order to find out the function of this isoform b in bone cells.

VITAMIN D ACTIVATION AND AI SECONDARY EFFECTS

In this study, SNPs in *VDR* and *CYP27B1* were associated with AIA, suggesting an important role of vitamin D in the undesirable joint symptoms[342]. *CYP27B1*, encodes for the key 1-hydroxylase enzyme, producing 1,25$(OH)_2$D.

VDR, in turn, encodes the receptor for vitamin D. Both genes act downstream of 25(OH)D.

It is worth mentioning that no significant differences in 25(OH)D serum levels were found among genotypes for any SNP at 3 or 12 months of follow-up[342]. The $1,25(OH)_2D$, the only biologically active form of vitamin D has not been assessed in the B-ABLE cohort since the thigh regulation together with its short half-life and the circadian variation levels makes it a bad accurate means to estimate vitamin D status. Thus, when trying to diagnose vitamin D deficiency or to assess vitamin D stores, 25(OH)D is the preferred form[380].

Increasing vitamin D substrate via higher doses may increase the active hormone 1,25-dihydroxyvitamin D with resultant reduction in joint symptoms. However, this intervention would be insufficient for those patients with "unfavourable genotypes" for some SNPs in *CYP27B1* and *VDR*. As a result, normal serum concentrations of 25(OH)D and AIA could coexist in the same individual.

Such unfavourable genetic background could be a potential predictor for developing joint discomfort during AI therapy. The ability to anticipate these symptoms may encourage patient – physician communication, treatment compliance and immediate decision-making for the benefit of the patient.

THE CHALLENGES AHEAD OF THE STUDY

This work has contributed to the understanding of AI-associated secondary effects in postmenopausal women with breast cancer. However, several issues remain to be settled.

Identification of causal sequence variants

The sample size of the B–ABLE cohort is still limited in terms of finding strong correlations of genetic variants with modest effect and quantitative traits. Thus, further research is needed to validate the described SNPs as predictors of AI–associated undesirable symptoms. Replication of our discoveries in larger and independent cohorts would be thus highly valuable.

In addition, association does not mean causality. Extending these initial associative findings to identification of the true causal variants involves searching through DNA regions in the vicinity of disease–associated SNPs. Those variants in functional elements including protein coding, regulatory and structural sequences are of true interest. We have started working on this challenging task: as a preliminary result, we have found a microsatellite polymorphism located at 528 base pairs upstream of the translation level of *CYP11A1*. In this regard, this polymorphism has been already associated to some other interesting phenotypes such as polycystic ovary syndrome[381].

Another key issue with respect to our findings relates to the fracture phenotype. Unfortunately, there are few data describing the heritability of osteoporotic fracture, mainly because recruiting adequate numbers of study subjects with fracture is difficult and expensive. Several studies have shown that a family history of fracture is a risk factor for fracture, and importantly, this is independent of BMD[382,383]. Some others have even noticed that the genes involved in fracture may be separate to those influencing BMD[384]. Therefore, finding the genes responsible for BMD variation does not necessarily identify genes causative for fracture. Incident fractures thorough AI therapy are being recruited in the B–ABLE study and the

corresponding analysis will be done when an appropriate sample size is achieved.

Sex steroid hormone levels in AI–treated women

AI are considered to cause estrogen level suppression to undetectable levels. However, reliable evidences point to a higher than expected interindividual variability in the estrogen levels during AI therapy[371,373]. For the purpose of exploring this issue, serum samples of B–ABLE cohort patients are currently being collected.

Taking advantage of the previous goal, we also considered of strong interest to assess circulating androgen levels in B–ABLE patients. It is likely that androgens are able to produce potent effects in musculoskeletal tissue without influence of the aromatase enzyme[385–387]. Thus, androgens could be clinically relevant, for instance, in the course of treatments that modify their availability, and particularly when AI are used. Therefore, it would be interesting to obtain information on the influence of AI on the serum levels of pre–androgens and androgens. Specifically, to clarify if the secondary effects of AI are affected by androgen levels, before and during AI treatment.

At present, 50 serum samples have been tested for estradiol, estrone, estrone conjugates, testosterone and androstenedione levels at baseline and at 3 months of AI therapy. In accordance with our hypothesis, a great variability in hormone levels is expected. Upcoming analyses will examine potential connections between the polymorphic variants described in our study, the hormone levels and the intensity of musculoskeletal effects of AI therapy. The discovery of a relationship between these factors may shed light on the

causality of AI secondary effects.

Biochemical assessment of bone remodelling and cartilage degradation during AI treatment

Biochemical monitoring of bone metabolism depends upon measurement of enzymes and proteins released during bone formation and of degradation products produced during bone resorption. The field of bone turnover markers (BTM) has developed considerably in the past decade and various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton[388]. The combined use of BMD and TBS measurement as well as bone markers is likely to improve the assessment of the risk of fractures[389].

In several of the trials analysing AI effects on bone, markers of bone resorption and formation were increased during AI therapy[246,390,391]. By contrast, BTM remained constant or decreased with tamoxifen therapy[390,391]. In order to address this point, B-ABLE also recruits information of BTM. Thus, P1NP (type 1 procollagen), NTX (urine N-telopeptide), CTX, (C-telopeptide), OC (osteocalcin) and BALP (serum bone specific alkaline phosphatase) are being measured at each follow-up visit. Our preliminary analyses agree with the previous reported results: both resorption and formation BTM increased at both 3 and 12 months of AI therapy. By contrast, in the group of patients receiving BP, a decrease of all BTM has been detected. These analyses will be repeated when more sample size is achieved.

Cartilage destruction leads to an accumulation of breakdown products in the synovial fluid. These are released into the circulation and ultimately filtered and excreted, or broken down in vivo[392]. Analysis of body fluids can provide

information regarding the health, or turnover of the cartilage, prior to the development of gross pathology, or can highlight any metabolic changes attributable to the treatment being studied. In this regard, the low-estrogen state in ovariectomized animals induces acceleration of cartilage turnover, presumably from their low-estrogen state[393]. Thus, P2NP (type 2 procollagen), C2M and C3M have been already measured in a group of patients of the B-ABLE cohort. These data, which are currently under evaluation, will explore the possibility that the AI-induced estrogen deficiency is contributing to AIA from lack of cushioning in the joints. Assessment of cartilage and/or bone turnover markers may bring the possibility for physicians to predict which patients are eligible for therapy intervention before the manifestation of the pathologies (that is, high arthralgia intensity, with it associated risk of therapy discontinuation and/or bone mineral density loss with its associate fracture risk).

Chapter 4

CONCLUSIONS

Si realmente viéramos al Universo, tal vez lo entenderíamos

Jorge Luis Borges

1. In actual clinical practice, AI treatment in women with breast cancer leads to joint pain, which ultimately affects treatment compliance.

2. In actual clinical practice, AI treatment in women with breast cancer leads to an accelerated bone mineral density loss and bone microarchitecture deterioration.

3. In actual clinical practice, those patients with breast cancer on AI treatment receiving oral BP experience significant increases in bone mineral density and a positive maintenance of bone microarchitecture.

4. Genetic variants in genes involved in estrogen and vitamin D metabolic pathways play a key role in the risk to suffer AI–associated musculoskeletal side effects:

    4.1 Genetic variants in the *CYP17A1*, *VDR*, and *CYP27B1* genes can help to predict the risk of AIA.

    4.2 Genetic polymorphisms of the *CYP11A1* gene are associated with AIBL.

5. The bone possess all the enzymes involved in the steroid–synthesis pathway, raising the possibility that this tissue can synthesize androgens and estrogens independently of serum steroid precursors.

6. Monitoring of bone health as well as calcium and vitamin D supplementation are essential for the clinical management of the detrimental effects of AI on bone tissue.

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