Medical ovariectomy in menopausal breast cancer patients with high testosterone levels: a further step toward tailored therapy

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Abstract

Five years of adjuvant therapy with anti-estrogens reduce the incidence of disease progression by about 50% in estrogen receptor-positive breast cancer patients, but late relapse can still occur after anti-estrogens have been discontinued. In these patients, excessive androgen production may account for renewed excessive estrogen formation and increased risks of late relapse. In the 50% of patients who do not benefit with anti-estrogens, the effect of therapy is limited by de novo or acquired resistance to treatment. Androgen receptor and epidermal growth factor receptor overexpression are recognized mechanisms of endocrine resistance suggesting the involvement of androgens as activators of the androgen receptor pathway and as stimulators of epidermal growth factor synthesis and function. Data from a series of prospective studies on operable breast cancer patients, showing high serum testosterone levels are associated to increased risk of recurrence, provide further support to a role for androgens in breast cancer progression. According to the above reported evidence, we proposed to counteract excessive androgen production in the adjuvant setting of estrogen receptor-positive patients and suggested selecting postmenopausal patients with elevated levels of serum testosterone, marker of ovarian hyperandrogenemia, for adjuvant treatment with a gonadotropins-releasing hormone analogue (medical oophorectomy) in addition to standard therapy with anti-estrogens. The proposed approach provides an attempt of personalized medicine that needs to be further investigated in clinical trials.

Introduction

Breast cancer patients with early-stage estrogen receptor (ER)-positive tumors are commonly treated with anti-estrogens, tamoxifen and aromatase inhibitors, to prevent cancer recurrence. Five years of adjuvant therapy significantly improve the outcomes of these patients compared to none therapy (Smith *et al.* 2014, Sestak & Cuzick 2015, Sestak *et al.* 2016), but, despite their effectiveness, adjuvant hormonal treatments encounter two major clinical problems: high rate of de novo or acquired resistance to anti-estrogens, that prevents about

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50% of patients to benefit with the therapy (Rechoum et al. 2014, Fuji et al. 2014, Ciupek et al. 2015) and occurrence of late relapse after anti-estrogens have been discontinued (Smith et al. 2014, Sestak & Cuzick 2015, Sestak et al. 2016).

Available evidence suggests that high levels of serum testosterone are risk factors for progression of breast cancer (Secreto & Zumoff 1983, Berrino et al. 2005, Micheli et al. 2007, Emond et al. 2011, Secreto 2012a, Secreto & Zumoff 2012) and that they may be involved in the occurrence of late relapse (Micheli et al. 2007). Furthermore, they are implicated in the development of endocrine resistance by stimulation of androgen receptor (AR) and epidermal growth-factor receptor (EGFR) overexpression (Fuji et al. 2014, Rechoum et al. 2014, Ciupek et al. 2015).

In a recently published article (Secreto et al. 2016), we proposed a novel approach to prevent breast cancer by administering a gonadotropins-releasing hormone (GnRH) analogue to healthy postmenopausal women with high levels of testosterone, marker of ovarian hyperandrogenemia. Similarly, in this paper, we proposed (i) to select ER-positive postmenopausal breast cancer patients based on high levels of serum testosterone and (ii) to inhibit excessive testosterone production by administration of a GnRH analogue (medical oophorectomy) in addition to standard adjuvant therapy with anti-estrogens.

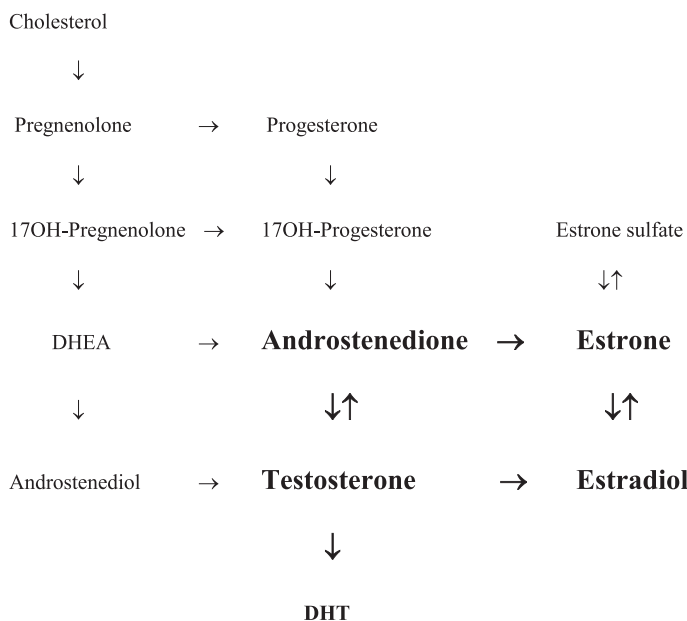In the next section, we have discussed the role of androgen excess in breast cancer development, the association of high testosterone levels with risk of disease progression, the role of androgen excess in late relapse and in resistance to endocrine therapy, the postmenopausal ovary as source of excessive testosterone production, subsequently we have proposed the statement of our proposal.

Discussion

Role of androgen excess in breast cancer development

The androgen excess theory, developed in studies by our and other groups over the last 45 years, states that high circulating levels of androgens represent a marker of increased risk of breast cancer in healthy women and of increased risk of relapse in women with breast cancer (Secreto 2012b). The mammary gland acts as an important para-endocrine organ capable of synthesizing active steroids from inactive or less active precursors holding the enzymatic supply for converting either one steroid into another of the same family (e.g. testosterone to dihydrotestosterone, estrone to estradiol) or one into a different family (testosterone to estradiol, androstenedione to estrone). A simplified scheme of the biosynthetic pathway of sex steroids and the related enzymes are reported in both Fig. 1 and in Table 1, respectively.

In ER-positive tumors, androgen excess operates through the increasing conversion into estrogens which are the final stimulators of cancer growth. The finding of a significant relationship of blood testosterone levels,

Cholesterol

↓

Pregnenolone   →   Progesterone

↓   ↓

17OH-Pregnenolone   →   17OH-Progesterone   Estrone sulfate

↓   ↓   ↓↑

DHEA   →   **Androstenedione**   →   **Estrone**

↓   ↓↑   ↓↑

Androstenediol   →   **Testosterone**   →   **Estradiol**

                       ↓

                    DHT

Figure 1

Biosynthetic pathway of sex steroids. The androgen to estrogen conversion is shown in bold to emphasize that estrone and estradiol can only derive from androstenedione and testosterone, respectively. DHT originates from testosterone and cannot be transformed into estrogens. DHT = dihydrotestosterone. Reproduced, with permission, from Secreto (2012a).

Table 1 Enzymes involved in sex steroid production. Reproduced, with permission, from Secreto (2012a).

Enzyme	Activity

Aromatase	Irreversible aromatization of:
	  Androstenedione to estrone
	  Testosterone to estradiol

17β-HSD* family	
17β-HSD5	17β-reduction of androstenedione to testosterone
17β-HSD1	17β-reduction of estrone to estradiol

17β-HSD2	17β-oxidation of:
	  Testosterone to androstenedione
	  Estradiol to estrone

Sulfatase	Conversion of estrone sulfate to estrone
Sulfotransferase	Conversion of estrone to estrone sulfate
5α-reductase	5α-reduction of testosterone to dihydrotestosterone

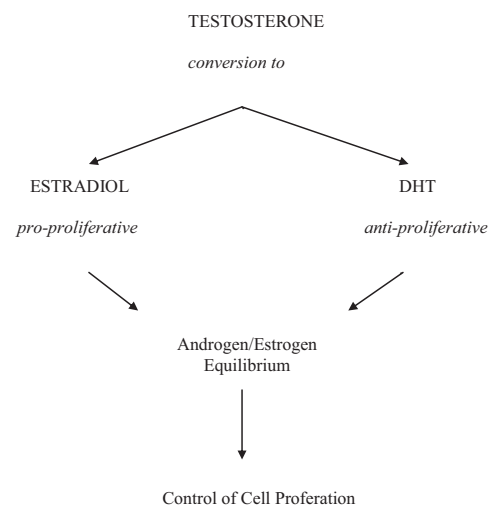*17β-hydroxysteroid dehydrogenase.

but not of estradiol levels, with the ER content of tumors (Secreto et al. 2009, 2011) supports this mechanism. The observation that testosterone stimulates development of ER-positive tumors has been previously described in two publications by our group (Secreto 2012a, Secreto et al. 2016). In brief, inside the cancer cell, testosterone is converted into its two biologically active metabolites: (i) estradiol, which stimulates proliferation of breast epithelium by binding to ER, and (ii) dihydrotestosterone (DHT), which counteracts the proliferative effect of estradiol by binding to AR. In hormone-dependent tumors, the equilibrium between the pro-proliferative effect of estradiol and the anti-proliferative effect of DHT is still maintained, although at a higher level than in normal cells. Thus, in this condition, cancer growth remains under hormonal control for some time; however, the proliferative effect of estrogens ultimately prevails if the source of excessive androgen production is not removed (Fig. 2).

In addition to increased conversion into estrogens, androgen excess can stimulate breast cancer growth by both a direct bind to AR (Farmer et al. 2005, Doane et al. 2006) and an increase in the epidermal growth factor (EGF) production, a known stimulator of breast epithelial cells proliferation (Lippman et al. 1986, Borellini & Oka 1989), whose synthesis and function are under the control of androgens (Labows et al. 1979, Pascall 1997) (Table 2). EGF is synthesized in specialized apocrine glands (Labows et al. 1979, Pascall 1997) and in the apocrine epithelium of the human mammary gland (Collette et al. 1986, Boccardo et al. 2001, Parish et al. 2007). Apocrine cysts are commonly found in the human breast and large areas of apocrine metaplasia are frequently present in the breast of women with mammary cancer (Mazoujian et al. 1983,

Dixon et al. 1985, Wellings & Alpers 1987), suggesting increased local production of EGF.

High testosterone levels and risk of disease progression: epidemiological evidence

A number of epidemiological prospective studies carried out in postmenopausal healthy women consistently reported the association of both elevated levels

TESTOSTERONE

conversion to

ESTRADIOL   DHT

pro-proliferative   *anti-proliferative*

Androgen/Estrogen
Equilibrium

Control of Cell Proferation

Figure 2
Central role of testosterone in breast epithelial cells proliferation. Simplified scheme to describe the control of breast epithelial cells proliferation by testosterone through conversion into its two biological active metabolites: estradiol and dihydrotestosterone (DHT). The equilibrium between the pro-proliferative effect of estradiol and the anti-proliferative effect of DHT allows proper control of breast epithelium proliferation during a woman's life. In presence of androgen excess, the proliferative effect of estrogens will ultimately prevail if the source of excessive androgen production is not removed.

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Table 2 Principal mechanisms by which androgen excess can stimulate growth of different breast cancer sub-types.

Androgen excess

Tumor type	Mechanisms of growth stimulation
ER-positive (often also AR-positive)	Increased conversion of testosterone to estradiol
ER-negative/AR-positive	Direct binding of testosterone and DHT* to AR
ER-negative/AR-negative	Increased synthesis of epidermal growth factor[†]

*DHT, dihydrotestosterone; [†]this mechanism is active in several tumors type.

of androgens and estrogens with increased risk of ER-positive breast cancer (Key et al. 2002). In contrast, a limited number of observational studies evaluated the association of androgen levels with risk of recurrence in women with breast cancer. In a series of prospective studies (Berrino et al. 2005, Micheli et al. 2007, Secreto 2012a, Secreto & Zumoff 2012), we found that the frequency of disease progression was greater in patients with baseline high serum testosterone levels (higher than 0.40 ng/mL, the median value of the group) than that in patients with low levels (below the median value). In one of these studies (Berrino et al. 2005), the use of diet to lower the circulating testosterone reduced the frequency of disease progression, suggesting that correction of hyperandrogenemia improved the outcomes of these patients. In a different observational study (Micheli et al. 2007) the authors have detected a better outcome for the low testosterone group than that for the high testosterone group, which became evident after the third year of follow-up. Furthermore, they have observed a constant and progressive increase in cancer survival in breast cancer patients at low testosterone serum levels during the 15-year follow-up period. Interestingly, in this low serum testosterone level group, the event-free survival at 10 years was 70% of the baseline group, which was only modestly declined at 15 years to 67%. On the contrary, event-free survival in the high testosterone group was already lower than that in the previous group at 10 years of follow-up, with only 52% event-free survival, reaching a very low 36% at 15 years of follow-up. The finding showed that after 10 years of follow-up virtually no further recurrence appeared in the low testosterone group whereas new events continued to emerge in the high testosterone group, suggesting that high testosterone levels at baseline may be regarded as a marker and player of late relapse. Confirmatory evidence that a high testosterone level is a factor of risk of breast cancer progression has been obtained in the largest of our prospective studies on 361 ER-positive postmenopausal patients. After 5.6 years of follow-up, we found that the rate of recurrence was almost double

in the high testosterone group (14.7%) than that in the low testosterone group (7.9%) ($P=0.037$) (Secreto 2012a, Secreto & Zumoff 2012). Data from a study by Emond et al. (2011) reported higher risk of cancer recurrence in patients with elevated levels of testosterone than that with normal levels. These study results are consistent with our findings. In contrast, Rock et al. (2008) found that increased risk of relapse was only associated with elevated levels of estradiol.

In contrast to increased risk of relapse observed in early breast cancer, excessive androgen production appeared to be a marker of favorable response to ovariectomy in metastatic disease (Secreto & Zumoff 1983). An explanation of this paradoxical effect is that androgen excess stimulates cancer progression and that ovariectomy achieves remission of metastases by removing the source of excessive androgen production. If ovariectomy improves the remission-rate of metastases by lowering androgen levels in patients with advanced disease then suppression of excessive ovarian androgen production in early disease should decrease the risk of future recurrence. Our reasoning is supported by the evidence produced by our team that remission of metastases after oophorectomy was more frequent and of longer duration in patients with high levels of urinary androgen than that with normal levels independently from the menopausal status (Grattarola 1976, Secreto et al. 1984). We have also observed that short-term cycles and low doses of dexamethasone were efficacious to inhibit adrenal androgen production when androgen levels started to rise again after ovariectomy. The combined treatment ovariectomy+dexamethasone obtained the best outcome (Table 3).

Androgen excess as a factor of risk of late relapse and of resistance to anti-estrogens

In the adjuvant therapy of ER-positive tumors, 5 years of treatment with anti-estrogens reduces the incidence of disease progression by about 50%, however, the disease progression can still occur after the therapy has been

Table 3 Incidence of objective remission after oophorectomy alone or in combination with dexamethasone (DXM) in metastatic breast cancer patients, according to normal or above normal urinary androgen (testosterone and/or androstanediol) excretion values. Adapted, with permission, from Secreto et al. (1984). *Reviews on Endocrine-Related Cancer* **14** (Supplement) p55.

Patients and treatment	Number of patients	Remission	
		N*	%
Normal androgen excretors Oophorectomy alone	61 (8)*	16 (2)	26.2
High androgen excretors Oophorectomy alone	46 (6)	25 (3)	54.3
High androgen excretors Oophorectomy+DXM	26 (3)	24 (3)	92.3

*Number of postmenopausal patients in parenthesis.

discontinued (Smith et al. 2014, Sestak & Cuzick 2015, Goss et al. 2016). Extending adjuvant treatments for additional 5 years efficiently reduces the incidence of late relapse but is associated with increased risk of adverse side effects of anti-estrogens (Davies et al. 2013, Goss et al. 2016, Sestak et al. 2016). Ten years of adjuvant treatments should be proposed only to patients at increased risk of late relapse, who will probably benefit with it, and should be avoided to the majority of patients for whom extended therapy will be useless and potentially dangerous (Smith et al. 2014, Sestak & Cuzick 2015). Clinical and pathological parameters are commonly used as predictors of late recurrence. Multi-gene signatures might also be used to identify women at increased risk of late relapse but, actually, these biomarkers are not routinely adopted in the clinical practice (Smith et al. 2014, Sestak & Cuzick 2015). Thus, we have suggested that high testosterone levels might be regarded as an additional prognostic marker.

According to the mechanism proposed above on the role of androgen excess in stimulating ER-positive tumors, anti-estrogens are effective against the consequence of androgen excess, i.e. increased estrogen synthesis and activity. However, they are ineffective against the source of androgen excess itself, which persists during adjuvant treatments supporting both the constant estrogen formation and the risk of late relapse at therapy discontinuation.

The prolonged beneficial carryover effect observed after suspension of anti-estrogens (Davies et al. 2013, Smith et al. 2014, Sestak & Cuzick 2015) suggests that stimulation of cancer cells by renewed estrogen formation can require a long-time interval before inducing cancer progression. The conception of testosterone as predictor

of breast cancer relapse is corroborated by the findings by Micheli et al. (2007) that after 10 years since diagnosis of disease progression continued to occur almost exclusively in patients with high testosterone levels.

In the 50% of ER-positive patients who do not benefit with anti-estrogen therapy, the effectiveness of therapy is limited by de novo or acquired resistance to treatment partially due to the AR and EGFR biological action. AR and EGFR overexpression are well documented mechanisms of anti-estrogens resistance (Massarweh et al. 2008, DeAmicis et al. 2010, Osborne & Schiff 2011, Rechoum et al. 2014, Fuji et al. 2014, Ciupek et al. 2015), which suggest the involvement of androgens both as activators of the AR pathway and as stimulators of EGF synthesis and function. Discussing multiple mechanisms of endocrine resistance in detail (DeAmicis et al. 2010, Rechoum et al. 2014) is beyond the scope of our paper: we only wish to emphasize the role of androgens in this process and the need of inhibiting excessive androgen production in addition to administration of anti-estrogens.

The postmenopausal ovary as source of excessive testosterone production

In postmenopausal women, estrogen formation occurs in peripheral tissues by aromatization of adrenal androgen precursors (dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), androstenedione) and ovarian androgen precursors (testosterone, androstenedione) (Burger 2002). The ovary is an important source of testosterone and other androgens in postmenopausal women (Fogle et al. 2007). Synthesis takes place in the stromal tissue under the stimulus of the gonadotropin luteinizing hormone (LH) (Adashi 1994, Laughlin et al. 2000). Continuous stimulation by high LH levels can lead to stromal cell hyperplasia and hence increased levels of circulating testosterone (Adashi 1994, Lucisano et al. 1986, Sluijmer et al. 1998, Jongen et al. 2003). Ovarian stromal hyperplasia is a characteristic feature of breast cancer patients, recognized over 60 years ago by Sommers & Teloh (1952) in 80% of women who had died of breast cancer and subsequently confirmed in studies by our group that have documented high testosterone levels in patients with breast cancer and ovarian interstitial cell hyperplasia (Grattarola 1973, 1976, Secreto & Zumoff 1983, Grattarola et al. 1974). Extensive evidence suggests that high serum testosterone levels are a marker of ovarian hyperandrogenemia (Lucisano et al. 1986,

Adashi 1994, Sluijmer *et al.* 1998, Laughlin *et al.* 2000, Burger 2002, Jongen *et al.* 2003, Fogle *et al.* 2007) which can be corrected by oophorectomy (Adashi 1994, Sluijmer *et al.* 1998, Laughlin *et al.* 2000, Jongen *et al.* 2003, Fogle *et al.* 2007, Kotsopoulos *et al.* 2012). In a study of BRCA1 and BRCA2 mutation carriers, Kotsopoulos *et al.* (2012) observed a significant decrease in the risk of breast cancer in women who are oophorectomized after menopause than that in women in natural menopause and suggested that the reduction of circulating testosterone levels after oophorectomy might account for its protective effect.

Statement of our proposal

In consideration of the data included in this report, we believe that there is enough evidence to support the development of a randomized clinical trial to test the hypothesis of whether the addition of GnRH analogues to anti-estrogens improves outcomes of postmenopausal patients with high testosterone levels. In these women treated with GnRH analogues, circulating testosterone should be regularly checked and the treatment interrupted when testosterone values decrease well under the cut-off value that separates elevated from normal levels (it may vary from population to population, from laboratory to laboratory, from analytical method to analytical method). Treatment should be then resumed when testosterone levels rise again after the initial decrease. The evaluation of serum testosterone levels should continue at regular time intervals for several years after that therapy with anti-estrogens has been discontinued and GnRH analogues should be administered when necessary, i.e. whenever testosterone levels rise again.

A limitation of our proposal is that the method for testosterone measurement is not standardized and actually there is not a general agreement about the normal values of testosterone in the general population. The choice of the cut-off value which more efficiently separates high from normal levels is an important issue and must take several variables into account and should be tested on a clinical trial. Actually, liquid chromatography-tandem mass spectrometry (LC–MS/MS) is the preferred method for measurement of low testosterone concentrations in women (Ketha *et al.* 2014). The LC–MS/MS technique permits simultaneous quantification of several steroids in a single run thereby allowing the organization of a supplementary hormonal study aimed to improve the knowledge on the role of sex steroids in breast cancer recurrence and resistance to anti-estrogens.

Tolerability and side effects of GnRH analogues in postmenopausal women have been discussed in our recently published paper on breast cancer prevention (Secreto *et al.* 2016). Here, we synthetically report that GnRH analogues are well tolerated and are not associated to significant side effects, especially no hot flushes, according to the few studies that examined the effect of medical oophorectomy at this age (Crighton *et al.* 1989, Hughes *et al.* 1991). In particular, inhibition of the synthesis of the gonadotropin follicle-stimulating hormone (FSH) does not influence the synthesis of estrogens in the adipose tissue (Folkerd *et al.* 1982, Bulun *et al.* 2005), which is the source of estrogen production at postmenopausal age. In a study of adjuvant therapy in premenopausal breast cancer patients, Pagani *et al.* (2014) found that 'the adverse-event profiles of exemestane plus ovarian suppression and tamoxifen plus ovarian suppression were similar to those seen in postmenopausal women'. Overall, co-administration of GnRH analogues should not worsen the adverse effects of anti-estrogens, in particular should not increase the risk of bone-related toxic effects associated to aromatase inhibitors, but the scanty information available on medical oophorectomy side effects in postmenopausal women require close follow-up of the treatment.

Conclusion

The aim of this report is to propose to administer GnRH analogues in addition to anti-estrogens to ER-positive postmenopausal patients with high testosterone levels to increase both disease-free survival and total survival in these patients. The scope of this article is to open innovative avenues for breast cancer clinical research in the rather unexplored field of the role of androgens in breast cancer development.

Suppression of estrogen synthesis and activity is the objective of adjuvant anti-estrogen hormonal therapy. We consider that such an approach is incomplete in patients with high testosterone levels and we propose to complete the treatment through the correction of the basic endocrine abnormality responsible of excessive androgen production. After the discontinuation of anti-estrogens, renewed estrogen synthesis is fast and abundant in patients with a severe degree of ovarian stromal hyperplasia, leading to disease progression in a short time. In patients with a mild degree of ovarian stromal hyperplasia, estrogen synthesis will be slower and cancer relapse will require a longer time, explaining the observed anti-estrogen

Anti-estrogens administration→ suppression of estrogen synthesis and/or activity

Anti-estrogens interruption→ renewed estrogen formation by androgen precursors

Ovarian stromal hyperplasia

Severe Degree	**Mild Degree**
↓	↓
Renewed Estrogen formation	Renewed Estrogen Formation
fast and abundant	slow and scarse
↓	↓
disease progression	disease progression
in a short time	in a long time
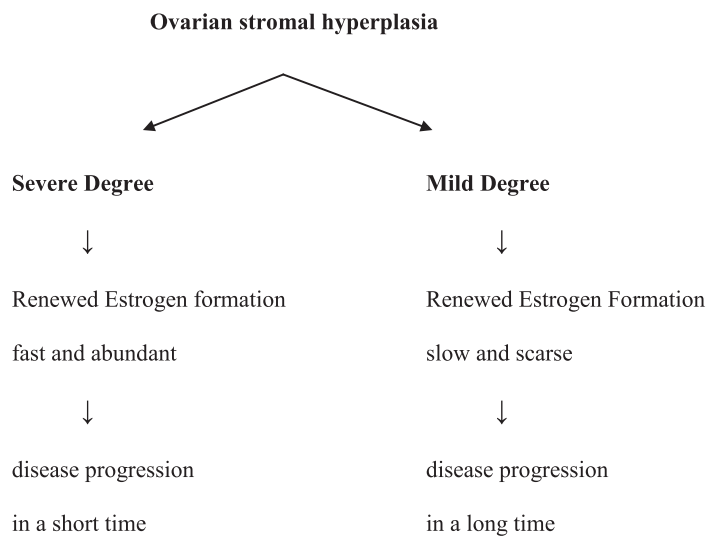
Figure 3
Renewed estrogen formation in patients with ovarian hyperandrogenemia (high testosterone levels) after that anti-estrogens are discontinued. Estrogen synthesis will be fast and abundant in patients with a severe degree of ovarian stromal hyperplasia, leading to disease progression in short time. Administering a GnRH analogue inhibits excessive ovarian androgen production and preserves from excessive estrogen formation thus restoring the normal androgen/estrogen equilibrium described in Fig 2.

beneficial carryover effect (Fig. 3). Administering a GnRH analogue inhibits excessive ovarian androgen production and preserves from excessive estrogen synthesis restoring the normal androgen/estrogen equilibrium.

This innovative therapeuthical approach does not completely neutralize the biologically important estrogen activity but proposes to restore the normal equilibrium between the proliferative effect of estradiol and the anti-proliferative effect of DHT in patients with high testosterone levels. This proposal is based on the administration of GnRH analogues in addition to anti-estrogens during the 5 years of standard adjuvant therapy to revert to normal ovarian androgen production. When anti-estrogens are suspended, a normal synthesis of estrogens will take place from a normal amount of androgen precursors, the stimulation of breast epithelial cells will not be unbalanced, and occurrence of late relapse will be prevented. In other words, normalization of estrogen production may achieve the same beneficial effects obtained by extending treatment with anti-estrogens but without their dangerous side effects. Preservation of the androgen/estrogen equilibrium can be monitored by measurement of testosterone levels at regular time intervals and can be corrected at any time by administration of GnRH analogues whenever it is necessary. In conclusion, we expect that lowering high testosterone levels and maintaining them low will counter endocrine resistance and will reduce the risk of early and late relapse, thus improving the outcomes obtained by anti-estrogens alone. As a final consideration, we emphasize that the proposed approach may provide a further step toward personalized therapy, which is the objective of modern oncology.

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