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.

Key Words
- breast cancer
- adjuvant therapy
- testosterone
- GnRH analogues
- medical ovariectomy

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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 2012*a*, 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 2012*b*). 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,

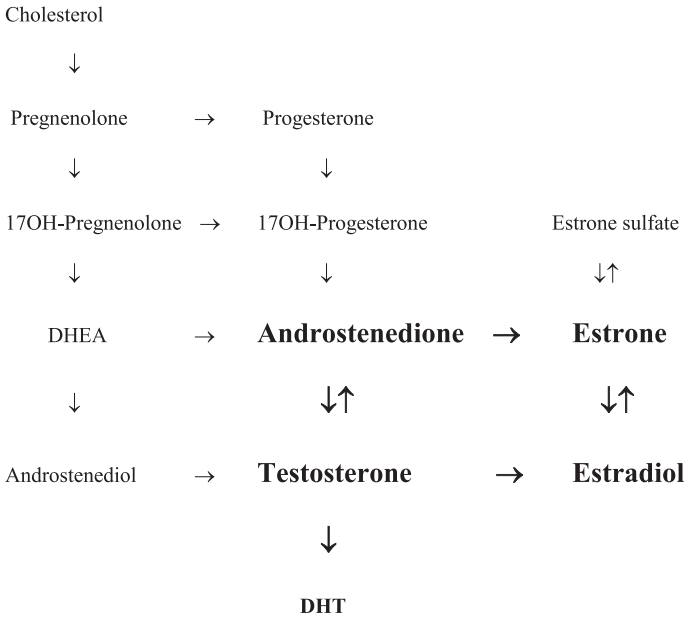

Figure 1
Biosynthetic pathway of sex steroids. The androgen to estrogen conversion is shown in bold to emphasize that estrone and estradiol can only derive from androstenedione and testosterone, respectively. DHT originates from testosterone and cannot be transformed into estrogens. DHT = dihydrotestosterone. Reproduced, with permission, from Secreto (2012*a*).

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

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 2012*a*, 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

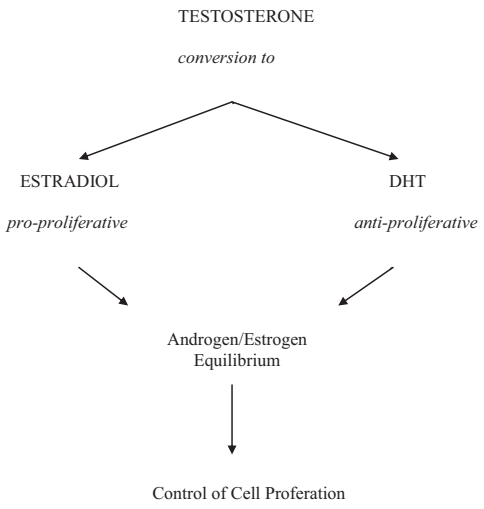

Figure 2
Central role of testosterone in breast epithelial cells proliferation. Simplified scheme to describe the control of breast epithelial cells proliferation by testosterone through conversion into its two biological active metabolites: estradiol and dihydrotestosterone (DHT). The equilibrium between 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 2012*a*, 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 2012*a*, 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

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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,

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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

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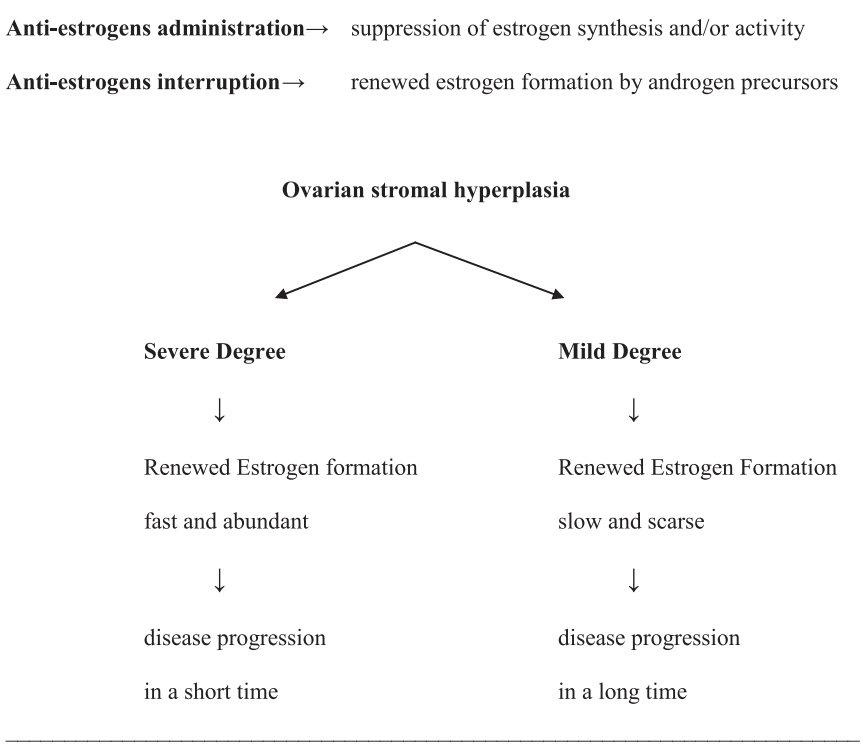

Figure 3
Renewed estrogen formation in patients with ovarian hyperandrogenemia (high testosterone levels) after that anti-estrogens are discontinued. Estrogen synthesis will be fast and abundant in patients with a severe degree of ovarian stromal hyperplasia, leading to disease progression in short time. 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.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Adashi EY 1994 The climacteric ovary as a functional gonadotropin-driven androgen-producing gland. *Fertility and Sterility* **62** 20–27.

Berrino F, Pasanisi P, Bellati C, Venturelli E, Krogh V, Mastroianni A, Berselli E, Muti P & Secreto G 2005 Serum testosterone levels and breast cancer recurrence. *International Journal of Cancer* **113** 499–502. (doi:10.1002/ijc.20582)

Boccardo F, Marenghi C, Ghione G, Pepe A, Parodi S & Rubagotti A 2001 Intracystic epidermal growth factor level is predictive of breast-cancer risk in women with gross cystic disease of the breast.

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International Journal of Cancer **295** 260–265. (doi:10.1002/1097-0215(20010720)95:4<260::AID-IJC1044>3.0.CO;2-N)

Borellini F & Oka T 1989 Growth control and differentiation in mammary epithelial cells. *Environmental Health Perspectives* **80** 85–89.

Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, Martin R, Utsunomiya H, Thung S, Gurates B, *et al*. 2005 Regulation of aromatase expression in estrogen responsive breast and uterine disease: from bench to treatment. *Pharmacological Reviews* **57** 359–383. (doi:10.1124/pr.57.3.6)

Burger HG 2002 Androgen production in women. *Fertility and Sterility* **77** (Supplement 4) S3–S5. (doi:10.1016/S0015-0282(02)02985-0)

Ciupek A, Rechoum Y, Gu G, Gelsomino L, Beyer AR, Brusco L, Covington KR, Tsimelzon A & Fuqua SA 2015 Androgen receptor promotes tamoxifen agonist activity by activation of EGFR in ERα-positive breast cancer. *Breast Cancer Research and Treatment* **154** 225–237. (doi:10.1007/s10549-015-3609-7)

Collette J, Hendrick JC, Jaspar JM & Franchimont P 1986 Presence of alpha-lactalbumin, epidermal growth factor, epithelial membrane antigen, and gross cystic disease fluid protein (15,000 daltons) in breast cyst fluid. *Cancer Research* **46** 3728–3733.

Crighton IL, Dowsett M, Lal A, Man A & Smith IE 1989 Use of luteinizing hormone-releasing hormone agonist (leuprorelin) in advanced post-menopausal breast cancer: clinical and endocrine effects. *British Journal of Cancer* **60** 644–648.

Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, Abraham M, Medeiros Alencar VH, Badran A, Bonfill X, *et al*. 2013 Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet* **381** 805–816. (doi:10.1016/S0140-6736(12)61963-1)

De Amicis F, Thirugnansampanthan J, Cui Y, Selever J, Beyer A, Parra I, Weigel NL, Herynk MH, Tsimelzon A, Lewis MT, *et al*. 2010 Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells. *Breast Cancer Research and Treatment* **121** 1–11. (doi:10.1007/s10549-009-0436-8)

Dixon JM, Lumdsen AB & Miller WR 1985 The relationship of cyst type to risk factors for breast cancer and the subsequent development of breast cancer in patients with breast cystic disease. *European Journal of Cancer and Clinical Oncology* **21** 1047–1050. (doi:10.1016/0277-5379(85)90289-5)

Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C & Gerald WL 2006 An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. *Oncogene* **25** 3994–4008. (doi:10.1038/sj.onc.1209415)

Emond JA, Patterson RE, Natarajan L, Laughlin GA, Gold EB & Pierce JP 2011 Sex hormone concentrations and the risk of breast cancer recurrence in postmenopausal women without hot flashes. *Cancer Epidemiology, Biomarkers and Prevention* **20** 939–945. (doi:10.1158/1055-9965.EPI-10-1240)

Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, Macgrogan G, Bergh J, Cameron D, Goldstein D, *et al*. 2005 Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* **24** 4660–4671. (doi:10.1038/sj.onc.1208561)

Fogle RH, Stanczyk FZ, Zhang X & Paulson RJ 2007 Ovarian androgen production in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **92** 3040–3043. (doi:10.1210/jc.2007-0581)

Folkerd EJ, Jacobs HS, van der Spuy Z & James VH 1982 Failure of FSH to influence aromatization in human adipose tissue. *Clinical Endocrinology* **16** 621–625. (doi:10.1111/j.1365-2265.1982.tb03179.x)

Fuji R, Hanamura T, Suzuki T, Gohno T, Shibahara Y, Niwa T, Yamaguchi Y, Ohnuki K, Kakugawa Y, Hirakawa H, *et al*. 2014 Increased androgen receptor activity and cell proliferation in aromatase inhibitor-resistant breast carcinoma. *Journal of Steroid Biochemistry and Molecular Biology* **144** 513–522. (doi:10.1016/j.jsbmb.2014.08.019)

Goss PE, Ingle JN, Pritchard KI, Robert NJ, Muss H, Gralow J, Gelmon K, Whelan T, Strasser-Weippl K, Rubin S, *et al*. 2016 Extending aromatase-inhibitor adjuvant therapy to 10 years. *New England Journal of Medicine* **375** 209–219. (doi:10.1056/NEJMoa1604700)

Grattarola R 1973 Androgens in breast cancer. I. Atypical endometrial hyperplasia and breast cancer in married premenopausal women. *American Journal of Obstetrics and Gynecology* **116** 423–428. (doi:10.1016/S0002-9378(15)31304-1)

Grattarola R 1976 Ovariectomy alone or in combination with dexamethasone in patients with advanced breast cancer and high levels of testosterone excretion. *Journal of the National Cancer Institute* **56** 11–16. (doi:10.1093/jnci/56.1.11)

Grattarola R, Secreto G, Recchione C & Castellini W 1974 Androgens in breast cancer. II. Endometrial adenocarcinoma and breast cancer in married postmenopausal women. *American Journal of Obstetrics and Gynecology* **118** 173–178. (doi:10.1016/0002-9378(74)90545-6)

Hickey TE, Robinson JL, Carroll JS & Tilley WD 2012 Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? *Molecular Endocrinology* **26** 1252–1267. (doi:10.1210/me.2012-1107)

Hughes CL Jr, Wall LL & Creasman WT 1991 Reproductive hormone levels in gynecologic oncology patients undergoing surgical castration after spontaneous menopause. *Gynecologic Oncology* **40** 42–45. (doi:10.1016/0090-8258(91)90083-H)

Jongen VHWM, Holleman H, van der Zee AGJ, Santema JG & Heineman MJ 2003 Ovarian stromal hyperplasia and ovarian vein steroid levels in relation to endometrioid endometrial cancer. *British Journal of Obstetrics and Gynaecology* **110** 690–695. (doi:10.1046/j.1471-0528.2003.02389.x)

Ketha H, Kaur S, Grebe SK & Singh RJ 2014 Clinical applications of LC–MS sex steroid assays: evolution of methodologies in the 21st century. *Current Opinion in Endocrinology, Diabetes and Obesity* **21** 217–226. (doi:10.1097/MED.0000000000000068)

Key T, Appleby P, Barnes I & Reeves G 2002 Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *Journal of the National Cancer Institute* **94** 606–616. (doi:10.1093/jnci/94.8.606)

Kotsopoulos J, Lubinski J, Lynch HT, Kim-Sing C, Neuhausen S, Demsky R, Foulkes WD, Ghadirian P, Tung N, Ainsworth P, *et al*. 2012 Oophorectomy after menopause and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Cancer Epidemiology, Biomarkers and Prevention* **21** 1089–1096. (doi:10.1158/1055-9965.EPI-12-0201)

Labows JN, Petri G, Hoelze E, Leyden J & Klingman A 1979 Steroid analysis of human apocrine secretion. *Steroids* **34** 249–258. (doi:10.1016/0039-128X(79)90077-1)

Laughlin GA, Barrett-Connor E, Kritz-Silverstein D & von Mühlen D 2000 Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo study. *Journal of Clinical Endocrinology and Metabolism* **85** 645–651. (doi:10.1210/jcem.85.2.6405)

Lippman ME, Dickson RB, Bates S, Knabbe C, Huff K, Swain S, McManaway M, Bronzert D, Kasid A & Gelmann EP 1986 Autocrine and paracrine growth regulation of human breast cancer. *Breast Cancer Research and Treatment* **7** 59–70. (doi:10.1007/BF01806790)

Lucisano A, Russo N, Acampora MG, Fabiano A, Fattibene M, Parlati E, Maniccia E & Dell'Acqua S 1986 Ovarian and peripheral androgen and oestrogen levels in post-menopausal women: correlations with ovarian histology. *Maturitas* **8** 57–65. (doi:10.1016/0378-5122(86)90008-3)

Massarweh S, Osborne CK, Creighton CJ, Qin L, Tsimelzon A, Huang S, Weiss H & Rimawi M 2008 Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. *Cancer Research* **68** 826–833. (doi:10.1158/0008-5472.CAN-07-2707)

Mazoujian G, Pinkus GS & Haagensen DE 1983 Immunochemistry of a gross cystic disease fluid protein (GCDFP-15) of the breast. A marker of apocrine epithelium and breast carcinomas with apocrine features. *American Journal of Pathology* **110** 105–112.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0251

Published by Bioscientifica Ltd.

McNamara KM, Moore NL, Hickey TE, Sasano H & Tilley WD 2014 Complexities of androgen receptor signalling in breast cancer. *Endocrine-Related Cancer* **21** T161–T181. (doi:10.1530/ERC-14-0243)

Micheli A, Meneghini E, Secreto G, Berrino F, Venturelli E, Cavalleri A, Camerini T, Di Mauro MG, Cavadini E, *et al*. 2007 Plasma testosterone and prognosis of postmenopausal breast cancer patients. *Journal of Clinical Oncology* **25** 2685–2690. (doi:10.1200/JCO.2006.09.0118)

Osborne CK & Schiff R 2011 Mechanisms of endocrine resistance in breast cancer. *Annual Review of Medicine* **62** 233–247. (doi:10.1146/annurev-med-070909-182917)

Pagani O, Regan MM & Francis PA 2014 Exemestane with ovarian suppression in premenopausal breast cancer. *New England Journal of Medicine* **371** 1358–1359. (doi:10.1056/NEJMc1409366)

Parish DC, Ghilchik MW, Day JM, Eaton J, Purohit A & Reed MJ 2007 Cytokines in human breast cyst fluid. *Journal of Steroid Biochemistry and Molecular Biology* **104** 241–245. (doi:10.1016/j.jsbmb.2007.03.021)

Pascall JC 1997 Post-transcriptional regulation of gene expression by androgens: recent observations from the epidermal growth factor gene. *Journal of Molecular Endocrinology* **18** 177–180. (doi:10.1677/jme.0.0180177)

Rechoum Y, Rovito D, Iacopetta D, Barone I, Andò S, Weigel NL, O'Malley BW, Brown PH & Fuqua SA 2014 AR collaborates with ERα in aromatase inhibitor-resistant breast cancer. *Breast Cancer Research and Treatment* **147** 473–485. (doi:10.1007/s10549-014-3082-8)

Rock CL, Flatt SW, Laughlin GA, Gold EB, Thomson CA, Natarajan L, Jones LA, Caan BJ, Stefanick ML, Hajek RA, *et al*. 2008 Women's Healthy Eating and Living Study Group. Reproductive steroid hormones and recurrence-free survival in women with a history of breast cancer. *Cancer Epidemiology, Biomarkers and Prevention* **17** 614–620. (doi:10.1158/1055-9965.EPI-07-0761)

Secreto G 2012*a* Endocrine classification of postmenopausal breast cancers. In *The Androgen-Excess Theory of Breast Cancer*, pp 80–109. Eds G Secreto & B Zumoff. Trivandrum, Kerala, India: Research Signpost. (available at: http://www.trnres.com/ebook.php)

Secreto G 2012*b* The androgen-excess theory of breast cancer. In *The Androgen-Excess Theory of Breast Cancer*, pp 47–70. Eds G Secreto & B Zumoff. Trivandrum, Kerala, India: Research Signpost. (available at: www.trnres.com/ebook.php)

Secreto G & Zumoff B 1983 Paradoxical effects associated with supranormal urinary testosterone excretion in premenopausal women with breast cancer: increased risk of postmastectomy recurrence and higher remission rate after ovariectomy. *Cancer Research* **43** 3408–3411.

Secreto G & Zumoff B 2012 Role of androgen excess in the development of estrogen receptor-positive and estrogen receptor-negative breast cancer. *Anticancer Research* **32** 3223–3228.

Secreto G, Oriana S & Recchione C 1984 Ovariectomy alone or in combination with dexamethasone in patients with advanced breast cancer and high levels of testosterone or androstanediol secretion. *Reviews on Endocrine-Related Cancer* **14** (Supplement) 55–58.

Secreto G, Venturelli E, Meneghini E, Greco M, Ferraris C, Gion M, Zancan M, Fabricio AS, Berrino F, Cavalleri A & Micheli A 2009 Testosterone and biological characteristics of breast cancers in postmenopausal women. *Cancer Epidemiology, Biomarkers and Prevention* **18** 2942–2948. (doi:10.1158/1055-9965.EPI-09-0540)

Secreto G, Meneghini E, Venturelli E, Cogliati P, Agresti R, Ferraris C, Gion M, Zancan M, Fabricio AS, Berrino F, *et al*. 2011 Circulating sex hormones and tumor characteristics in postmenopausal breast cancer patients. A cross-sectional study. *International Journal of Biological Markers* **26** 241–246. (doi:10.5301/JBM.2011.8883)

Secreto G, Sieri S, Agnoli C, Grioni S, Muti P, Zumoff B, Sant M, Meneghini E & Krogh V 2016 A novel approach to breast cancer prevention: reducing excessive ovarian androgen production in elderly women. *Breast Cancer Research and Treatment* **158** 553–561. (doi:10.1007/s10549-016-3901-1)

Sestak I & Cuzick J 2015 Markers for the identification of late breast cancer recurrence. *Breast Cancer Research* **17** 10. (doi:10.1186/s13058-015-0516-0)

Sestak I, Dowsett M, Ferree S, Baehner FL & Cuzick J 2016 Retrospective analysis of molecular scores for the prediction of distant recurrence according to baseline risk factors. *Breast Cancer Research and Treatment* **159** 71–78. (doi:10.1007/s10549-016-3868-y)

Sluijmer AV, Heineman MJ, Koudstaal J, Theunissen PH, de Jong FH & Evers JL 1998 Relationship between ovarian production of estrone, estradiol, testosterone, and androstenedione and the ovarian degree of stromal hyperplasia in postmenopausal women. *Menopause* **5** 207–210.

Smith IE, Yeo B & Schiavon G 2014 The optimal duration and selection of adjuvant endocrine therapy for breast cancer: how long is enough? *American Society of Clinical Oncology Educational Book* e16–e24. (doi:10.14694/EdBook_AM.2014.34.e16)

Sommers SC & Teloh HA 1952 Ovarian stromal hyperplasia in breast cancer. *Archives of Pathology* **53** 160–168.

Wellings SR & Alpers CE 1987 Apocrine cystic metaplasia: subgross pathology and prevalence in cancer-associated versus random autopsy breasts. *Human Pathology* **18** 381–386. (doi:10.1016/S0046-8177(87)80169-7)

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