Review

Clinical Cancer Research

Sex Hormones and Anticancer Immunity

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Abstract

The impact of sex hormones on anticancer immunity deserves attention due to the importance of the immune system in cancer therapy and the recognition of sex differences in immunity. Cancer is ultimately the result of failed immune surveillance, and the diverging effects of male and female sex hormones on anticancer immunity could contribute to the higher cancer incidence and poorer outcome in men. Estrogens and androgens affect the number and function of immune cells, an effect that depends on cell type, tumor microenvironment, and the age and reproductive status of the individual. Despite the recent progress in immuno-oncology, our current understanding of the interplay between sex hormones and anticancer immune responses is in its infancy. In this review, we will focus on the impact of sex hormones on anticancer immunity and immunotherapy. We will discuss the potential role of the changing hormone levels in anticancer immunity during aging and in the context of menopausal hormone therapies and oral contraception. We will review emerging data on sex differences in PD-L1 expression and potential biomarkers predictive for the efficacy of immune checkpoint inhibitors such as the microbiome and consider ongoing clinical trials evaluating the potential impact of hormone deprivation therapies to increase response to immune checkpoint inhibitors in breast and prostate cancer. Finally, we will point to areas of future research.

Introduction

Sex differences in cancer susceptibility and survival are well documented. Worldwide, men have a higher risk and mortality than women across various cancer types and races, with a few notable exceptions such as thyroid and gallbladder cancer (1). Obvious differences between males and females are sex chromosomes and sex hormones. Both influence self-renewal of target stem cell populations, the tumor microenvironment, and systemic determinants of carcinogenesis, such as cell metabolism and the immune system (2).

A great achievement in oncology in the last years was the recognition of the role of the immune system in cancer development, with the introduction of immunotherapy for a variety of cancer types such as melanoma, lung, and urinary tract cancers (3). It is accepted that cancer development is the result of failed immune surveillance as illustrated by animal models (4) and the increased cancer risk of immunosuppressed patients, as a consequence of organ transplant rejection prevention (5) or HIV infection (6). Tumors showing a strong immune cell infiltration ("inflamed") elicit an innate immune response but escape cell killing by cytotoxic T cells probably through immunosuppressive pathways activated by cancer cells, while in "immune-excluded" tumors, the development of an effective antitumor immune response is blocked by the retention of the immune cells in the tumor stroma. In contrast to the inflamed and immune-excluded tumor types, tumors devoid of T cells ("immune desert") do not activate the innate immune system in the first place (7).

Innate and adaptive immune responses are affected by both chromosomal and hormonal factors and differ between men and women (8). Women have overall stronger immune responses, as shown by the higher incidence of autoimmune diseases in women and data on vaccine and infection responses (8). The sex hormones estrogen, progesterone, and testosterone regulate a variety of cellular functions in both reproductive and nonreproductive tissues, which are yet insufficiently studied (2). Nearly all immune cells express receptors for these hormones (9–13) and many immune-related genes possess androgen receptor (AR)– and estrogen receptor (ER)–responsive elements in their promoters, which may underlie the sex differences in immune responses (14). These can depend on specific immune cell types and their location as well as hormone levels and density and distribution of their receptors. In fact, the same sex hormones can have both immune-stimulatory and inhibitory effects as a function of dose(s) and time(s) (15). A comprehensive review of the sex differences in immunity is beyond the scope of this article; for a recent review on the hormonal effects on immune cells, see ref. 8.

Estrogens and androgens have been shown to exert opposite effects on B and T cells, macrophages, neutrophils, and natural killer (NK) cells (10, 16–18). However, it is important to stress that these differences have been mostly studied in mouse models, and whether or not they apply directly to human cells and especially to patients with cancer is currently unknown.

Given that the field is yet developing, the scope of this review is to discuss what we know and what we need to learn about the impact of sex hormones in anticancer immunity in humans in various contexts, such as aging, menopausal hormone therapy, and oral contraception. There is currently considerable interest in cancer immunotherapies and predictive biomarkers of response. We will outline emerging sex differences in efficacy of immune

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checkpoint inhibitors and underlying possible causes, such as levels of PD-L1 expression. Ongoing clinical trials evaluating the potential impact of sex hormone deprivation therapies on response to immune checkpoint inhibitors in breast and prostate cancer will be considered. Finally, we will point to future areas of research such as the impact of pregnancy on anticancer immunity, the role of sex hormones on tumor dormancy, and clinical trial design tailored according to sex hormone levels and other sex differences.

Effect of Sex Hormones on Immunity as a Function of Age

The exponential increase of cancer incidence with age can be attributed to various factors including the accumulation of genetic mutations and changes in the immune system. An essential question to be addressed is how the changes in hormones over the lifespan affect various immune cell types and ultimately the clinical behavior and treatment outcome of various cancers.

Aging exerts a significant impact on estrogen and androgen levels in both males and females with some significant differences and commonalities. While estrogen levels decrease drastically only in women with menopause, there is a rather progressive decrease in androgens in both sexes beginning around the age of thirty (19–21). In turn, the aging process is itself enhanced by decreasing sex hormones levels, resulting for instance in age-related loss of muscle and bone mass and decline of various physiologic functions (22).

Variations in sex hormone levels can now be better appreciated, as a recently developed LC/MS-MS assay allows accurate measurement even of the low levels of testosterone in women (23). Women synthesize androgens and their precursors (e.g., dehydroepiandrosterone, DHEA) in the adrenal glands and ovaries (24) with premenopausal total testosterone levels corresponding to one-twentieth of those found in adult men (20, 25). In men, testosterone is reduced to dihydrotestosterone (DHT), which binds more avidly to the AR. In women, testosterone is converted into estradiol by the cytochrome P450 aromatase present in adipose tissue, skin, bone, and other organs (26). Some studies (27), but not others (28), have suggested that estradiol levels decrease in parallel with testosterone in aging men (Table 1). In general, considerable interindividual heterogeneity renders the establishment of age-dependent reference ranges for sex hormones in men and women challenging (27). In addition, an individual's true sex hormone status during lifetime is most likely determined by the combination of serum estradiol, testosterone, DHT, and sex hormone binding globulin (SHBG) levels, which determine the concentration of the biologically active free hormone, as well as body mass index (BMI) and sex hormone receptor activity (ref. 29; Table 1).

Aging in men is correlated with a more pronounced decline in total T- and B-cell numbers and a larger increase in senescent CD8 T effector memory cells compared with aging women (30, 31). In women, menopause is associated with an increase in proinflammatory IL1β, IL6, and TNFα levels and a reduction in the anti-inflammatory IFNγ levels. Monocytes and NK cells of aged women exhibit a proinflammatory phenotype and more robust cytotoxic activity, respectively, compared with those of aged men. For a recent comprehensive review on the effect of sex hormones on aging and immunity, see ref. 32.

Aging induces a state of chronic low-grade inflammation that has been named as "inflammaging" and which is believed to be the consequence of various inflammatory cytokines secreted by the increasing number of senescent cells in several organs. Multiple stimuli such as oxidative stress, DNA damage, telomere dysfunction, or environmental carcinogens can induce the senescence-associated secretory phenotype (SASP), which has been proposed to be the main origin of "inflammaging" in both aging and age-related diseases such as cancer (33).

Although not reported, it is likely that the physiologic decline in sex hormones during aging plays a role in cellular senescence, which could be of preventive and/or therapeutic significance. In fact, recent evidence from our laboratory indicates that, at least in dermal fibroblasts of both female and male individuals and in fibroblasts associated with skin cancer lesions, genetic or pharmacologic suppression of androgen signaling induces a SASP with associated tumor-enhancing properties, while androgen stimulation exerts opposite beneficial effects. Future work will have to further assess the clinical significance of the findings in the context of other cell types in which androgen signaling may exert a similar or opposite function (34).

Therapeutic Increase of Sex Hormone Levels and Cancer Immunity

The association between cancer risk and changes in sex hormone levels during aging implies that the effect of hormonal therapies on anticancer immunity should be further investigated. In women, menopausal hormone therapies (MHT) using a combination of estrogen and a progestogen (synthetic analogue of

Table 1. Differences in sex hormone levels in men and women during aging

	Age	Men	Women	References
Mean total testosterone (ng/dL)	25–54	469–553	23–45	(20, 25, 96)
	>55	469–475	19–20	
Mean free testosterone (ng/dL)	25–54	9–12	0.3–0.7	(20, 25, 96)
	>55	7–8.3	0.3	
Mean DHEAS (μg/dL)	25–54	151–286	126–276	(20, 96)
	>55	114–137	65–87	
Mean estradiol (pg/mL)	25–54	25.1–25.7	30–800	(28, 97)
	>55/postmenopausal	25.7–29.7	<20	
Mean free estradiol (pg/mL)	25–54	0.54–0.56	2.4–3.1	(28, 98)
	>55/postmenopausal	0.46–0.53	<0.5	

Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

progesterone) are associated with an increased cancer risk of hormone-responsive tissues such as breast (35), endometrium (36), and ovaries (37) to a varying extent, depending on the type of progestogen used (38). This does, however, not occur when estrogen is combined with a natural progesterone (38) and estrogen supplementation alone was reported to decrease (39) rather than increase breast cancer risk. In addition, the response of different tissues and cell types to MHT might also depend on the presence of concomitant risk factors such as obesity and the individual genetic background.

It was reported that MHT partially reverses the impact of aging on immunity by increasing B- and T-cell counts (40), and by decreasing levels of proinflammatory cytokines, TNFα and IL6, in postmenopausal women (41).

Likewise, various forms of hormonal contraception, mostly administered as oral estrogen–progestogen combinations, correlate with a small increase in breast cancer risk (analysis of data from 1.8 million women), which increases with longer durations of use (42). This occurs despite increased numbers of B and T cells (43), suggesting that the direct growth-stimulating effect of sex hormones on the epithelium of reproductive tissues might overweigh effects on the immune system. Experimental evidence for this hypothesis is, however, lacking and differences in types, doses, and duration of hormonal contraception might also alter its impact on immune responses. Long-term oral hormonal contraception is also correlated with increased risk of adenocarcinoma *in situ* of the cervix (44). While infection with human papillomavirus (HPV) and immunosuppression are established causes of cervix cancer, reports on the association between long-term hormonal contraception use and impaired virus clearance resulting in persistent HPV infections are inconsistent. It is possible that the persistence of only some oncogenic HPV types, such as HPV16, are associated with hormonal contraception exposure (45). Given that most women will be infected with HPV during their lifetime, a possible negative effect of hormonal contraception on immune responses against these viruses needs to be evaluated.

In contrast, hormonal contraception use is associated with a significant reduction of ovarian cancer risk (data from 45 epidemiologic studies; ref. 46) even in BRCA 1 or 2 gene mutation carriers (47). Also, a decreased risk for colorectal and endometrial cancer has been found in hormonal contraception users (48). The mechanisms behind these opposing effects of postmenopausal MHT and hormonal contraception on ovarian and endometrial cancer risk are currently unclear and it needs to be determined how the interference with physiologic sex hormone levels and cycles can selectively modulate cancer risk of different organs.

It has not been reported yet whether testosterone replacement therapy influences immune responses in aged men. However, given the growth-stimulatory effect of testosterone on prostate tissue, there is the clinical concern that testosterone therapy might increase prostate cancer risk. Although a meta-analysis of clinical trials of testosterone therapy compared with placebo found a nonsignificantly increased rate of prostate cancer and prostate-specific antigen (PSA) levels (49), various other reports indicated that testosterone therapy is safe (50, 51). These seemingly contradictory findings are explained by the "saturation model," which suggests that the prostate is most sensitive to testosterone effects at very low concentrations when ARs are receptive and becomes insensitive at higher levels. Once the AR is saturated, the presence of additional testosterone appears to have no further effect on prostate tissue (52).

Sex Differences in PD-L1 Expression and Response to Immune Therapies

The duration and magnitude of immune responses are tightly controlled by inhibitory immune checkpoints to avoid autoimmunity. These protective signaling pathways are often hijacked by tumors to escape immune surveillance (3). The currently best characterized immune checkpoints are CTL-associated protein 4 (CTLA-4), which is constitutively expressed in regulatory T cells (Tregs) and upregulated upon activation of naïve T cells; programmed cell death protein 1 (PD-1), which is found in T cells, B cells, and NK cells; and the PD-L1, a PD-1 ligand expressed in antigen-presenting cells and cancer cells (53). Some animal studies and emerging clinical evidence suggest a role for estrogens in upregulation of PD-1 and PD-L1 expression (54, 55), and for sex differences in the response to immune checkpoint inhibitors (56–58).

Female sex has been suggested as a negative predictive factor for response of patients with melanoma to anti–PD-1 therapy (56). One explanation for this finding might be the paucity of partially exhausted PD-1^{high}/CTLA-4–positive CD8 cells associated with response to combined checkpoint inhibition in women (59), while a hormone-mediated mechanism might also be important. However, in absence of preplanned subgroup analyses according to sex from large clinical trials or pooled analyses based on individual patient data, no definitive conclusions can be drawn yet.

Robust predictive biomarkers of good therapeutic response, beyond high PD-L1 expression, high tumor mutational burden, or the presence of tumor-infiltrating lymphocytes (TIL; ref. 60), are lacking.

The gut microbiome is emerging as a modulator of response to immune checkpoint inhibitors. In a recent study of patients with melanoma undergoing anti–PD-1 therapy, significant differences were found in the diversity and composition of the gut microbiome of responders compared with nonresponders. Patients with the most diverse microbiome were more likely to respond to immunotherapy, while antibiotic therapy was predictive of resistance to anti–PD-1 blockade. Fecal transplants from responders to germ-free or antibiotics-treated mice resulted in increased antitumor immunity with reduced tumor growth (61, 62).

Studies in mice and human have shown that the gut microbiome is affected by various factors including sex, age, diet, and obesity and itself also contributes substantially to sex differences in immunity (63, 64). For instance, transfer of gut microbiota from adult male mice to immature females changes the recipient's microbiota composition, leading to elevated testosterone levels and metabolomic changes, decreased inflammation, and protection from autoimmune type I diabetes (65). Also, gonadectomy and hormone replacement alters microbiota composition significantly in different mouse strains (63). Although the impact of a disturbed relationship between the host and the gut microbiome (dysbiosis) to carcinogenesis in various organs is well established (66), the cross-talk among sex hormones, microbiome composition, and immune system in men and women has as yet to be studied.

Also, obesity was positively correlated with overall survival in men with metastatic melanoma treated with immune checkpoint inhibitors, while no correlation was found in women (67). Although this kind of retrospective analysis has several

limitations, these findings are hypothesis-generating and insinuate possible biological and/or hormonal differences.

A comparison of the PD-1/PD-L1 expression in male versus female patients with cancer of different ages as well as in patients undergoing hormonal therapies is largely missing. Some small studies report an association between elevated PD-L1 expression and male sex (68, 69). Because in current clinical practice, PD-L1 positivity is mostly correlated with poor prognosis, but predictive of response to immune checkpoint inhibitors (60), sex differences in PD-L1 expression could partially account for the overall poorer prognosis of men and better response to immune checkpoint inhibitors. In fact, a recent meta-analysis of clinical trials of immune checkpoint inhibitors for various indications reported a significant survival advantage for men treated with anti–CTLA-4 or anti–PD-1 therapies compared with women (58). Even though these results are not based on individual patient data and the majority of the clinical trials are underpowered to detect clinically relevant sex differences in outcome and rarely report efficacy and toxicity according to sex, these results are thought-provoking. They hint at possible sex differences in the predominant immune escape mechanisms of cancers arising in men and women and indicate that the hormonal milieu might affect therapy response (Fig. 1).

A plethora of checkpoints attenuating (LAG3, TIM3) or stimulating (OX40, CD27) immune responses, respectively, have been identified and are being investigated as potential targets for immune therapies (70). In view of the recent data, the possibility that using different immunotherapy approaches in men and women could improve response rates merits further investigation. In addition, while immunotherapy-induced endocrinopathies are well documented, a possible impact on ovarian and testicular function has not been explored (71).

Sex Hormone Deprivation Therapies as Coadjuvants for Immune Therapies

Inhibition of estrogen or androgen signaling is a cornerstone in the treatment of hormone-dependent tumors such as breast and prostate cancers. Although it is difficult to evaluate their effects on anticancer immunity given that these therapies directly act on cancer cells, there is a considerable interest in exploring their impact in cancer immunotherapy in clinical trials (Table 2). It has to be noted that the question whether the immune response itself can be improved by either antiestrogen and antiandrogen therapies alone is not specifically addressed by these trials, and further analysis, for example, on tissue samples from such trials will be required to assess this point and extend similar trial design to other cancer types.

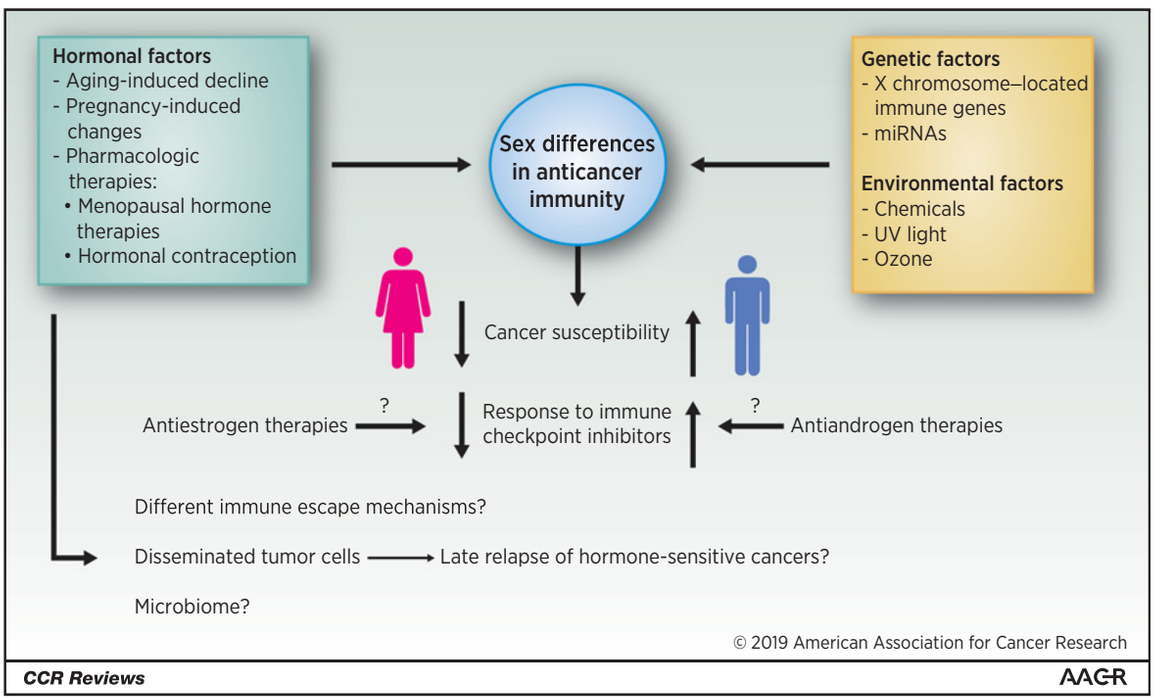

Figure 1.

Sex hormones, genetic factors, and environmental factors factors contribute to sex differences in anticancer immunity. The cross-talk between sex hormone signaling and genetic and environmental factors affects sex differences in innate and adaptive immunity. Variations of sex hormone levels during aging and pregnancy or due to pharmacologic intervention influence immune responses and can contribute to the sex disparities in oncology, with lower cancer susceptibility in female populations. Emerging data also suggest female sex as a predictor of poor response to immune checkpoint inhibitors. Clinical trials are currently evaluating whether antiestrogen or antiandrogen therapies could improve responses to such therapies. It remains to be determined whether these observed sex differences in immunotherapy responses are possibly due to differences in the predominant immune escape mechanisms in tumors arising in men and women. An effect of sex hormones on disseminated tumor cells and the late relapse of hormone-sensitive malignancies such as breast and prostate cancer as well as on the microbiome needs to be investigated.

Table 2. Clinical trials combining antiestrogen or androgen deprivation therapy with immunotherapies in breast and prostate cancer, respectively

Indication	Drugs	Phase	Study ID	Number of participants, primary endpoints	Study completion date
ER^+ $HER2^-$ BC	Exemestane + tremelimumab Exemestane + durvalumab	Phase I Phase II	NCT02997995 ULTIMATE	$N = 240$, pathologic CR	Sep 2020
ER^+ BC	Letrozole + pembrolizumab + palbociclib	Phase II	NCT02778685	$N = 22$, ORR	Feb 2019
HR^+ $HER2^-$ BC	Tamoxifen/fulvestrant/exemestane + atezolizumab + targeted therapies	Phase I/II	NCT03280563 MORPHEUS	$N = 111$, ORR	Oct 2022
HR^+ $HER2^-$ BC, premenopausal	Exemestane + leuprolide (GnRH analogue) + pembrolizumab	Phase I/II	NCT02990845	$N = 25$, PFS	Dec 2019
HR^+ IBC	Tamoxifen/aromatase inhibitor/LHRH agonist (physician's choice) + pembrolizumab	Phase II	NCT02971748	$N = 37$, DFS	Jan 2020
HR^+ BC or TNBC	Antiestrogen + pembrolizumab vs. pembrolizumab + doxorubicine	Phase II	NCT02648477	$N = 56$, safety, ORR	Sep 2019
AR^+ TNBC	Pembrolizumab + enobosarm (selective AR modulator)	Phase II	NCT02971761	$N = 29$, safety, ORR	Oct 2019
mCRPC	Enzalutamide + atezolizumab vs. enzalutamide	Phase III	NCT03016312	$N = 730$, OS	Jul 2022
mCRPC	Enzulatamide + pembrolizumab	Phase II	NCT02312557	$N = 58$, PSA response	Jan 2019
mCRPC	Enzalutamide + pembrolizumab vs. pembrolizumab	Phase II	NCT02787005 KEYNOTE 199	$N = 370$, ORR	Mar 2020
mCRPC	Enzalutamide + PROSTVAC-F/V-TRICOM vs. enzalutamide	Phase II	NCT01867333	$N = 57$, TTP	Jan 2019
mCRPC	Abiraterone acetate (CYP17 inhibitor) + prednisone + ipilimumab	Phase I/II	NCT01688492	$N = 57$, PFS, safety	Sep 2019
CSPC	Enzalutamide (AR antagonist) + PROSTVAC-F/V-TRICOM vs. enzalutamide	Phase II	NCT01875250	$N = 38$, tumor growth	July 2019
CSPC, adjuvant or after recurrence	Degarelix (GnRH antagonist) + ipilimumab	Phase II	NCT02020070	$N = 16$, PSA response	Dec 2019
Localized PC, neoadjuvant	Degarelix + cyclophosphamide + GVAX vs. degarelix	Phase I/II	NCT01696877	$N = 29$, $CD8^+$ T-cell infiltration, adverse events	May 2019

Abbreviations: AR^+, androgen receptor positive; BC, breast cancer; CR, complete response; CSPC, castration-sensitive prostate cancer; DFS, disease-free survival; HR^+: hormone receptor positive; IBC, inflammatory breast cancer; mCRPC, metastatic castration-resistant prostate cancer; NA, not available; ORR, overall response rate; OS, overall survival; PC, prostate cancer; TNBC, triple-negative breast cancer; TTP, time to progression.

Antiestrogen therapies

Tamoxifen and fulvestrant, a selective modulator and degrader of estrogen receptor, respectively, affect antigen presentation. *In vitro* and mouse experiments have shown a 2–3-fold increased expression of hormonally regulated tumor antigens such as α-Lactalbumin in ER-positive breast cancer cells treated with tamoxifen or fulvestrant (72). This upregulation in antigen expression is correlated with increased anticancer immunity given that tumor-bearing mice respond to treatment with antigen-specific lymphocyte transfer (72). Tamoxifen stimulates neutrophil activity *in vitro* and *in vivo* through modulation of sphingolipid biosynthesis (73) and was shown to diminish the number of immunosuppressive myeloid-derived suppressor cells (MDSC) and increase the population of effector and cytotoxic T cells that infiltrated the tumor in a mouse model of ERα-negative ovarian cancer (74).

The aromatase inhibitor letrozole significantly reduces the number of Tregs in human breast cancer tissue, which is correlated with therapy response (75).

These results suggest a potential role for antiestrogen therapies in enhancing the efficacy of immunotherapies and early-phase clinical trials are testing this hypothesis in hormone receptor–positive breast cancers (Table 2).

Androgen deprivation therapies

Similar immune-stimulatory effects were reported with the suppression of androgen signaling (76, 77). Immune cells isolated from men with androgen deficiencies produce more proinflammatory cytokines such as IL1β, IL2, and TNFα when stimulated with lipopolysaccharides (LPS) (78), which is reverted upon testosterone therapy (78–80). Androgen deprivation therapy (ADT), standard of care in prostate cancer, induces expansion of naïve T cells and increases T-cell responses, an effect observed from 1 to 24 months (81). Histologically, ADT is associated with a strong T-cell and macrophage infiltration into the prostate after one week of treatment (82, 83). Several studies demonstrated that ADT enhances susceptibility of AR-overexpressing prostate cancer cells to immune-mediated T-cell killing through improved immune recognition (84, 85). Emerging clinical data also reveals that ADT enhances the efficacy of various immunotherapies including immune checkpoint blockade (86) and cancer vaccines such as sipuleucel-T (87) and PROSTVAC (88).

Clinical trials combining ADT with abiraterone acetate, which inhibits androgen synthesis in the adrenals, and enzalutamide, an AR ligand–competitive antagonist with different immunotherapies, are ongoing. These combination therapies might improve the rather poor response rate of patients with prostate cancer to immune checkpoint inhibitors (Table 2).

Animal experiments (89) and data from a phase II clinical trial (88) in prostate cancer suggest that sequential therapy with administration of immunotherapy before rather than after or concomitantly to ADT could improve therapeutic responses. The optimal timing and duration of such therapies remain, however, to be determined, given that another phase II trial did not show any differences between sequential and concomitant enzalutamide therapy in response to sipuleucel-T (90).

Conclusions and Areas of Future Research

Despite the impressive achievements in the field of immuno-oncology, our understanding of the interplay between sex hormones and anticancer immunity is lagging behind. The immune

system of females and males evolves in a different hormonal environment resulting in distinct immune responses that vary with the aging-related decline in sex hormones. At the same time, genetic factors such as localization of many immune-related genes and the miRNAs implicated in their control on the X chromosome, are also likely to contribute to the observed sex disparities in immunity (91). In addition to investigating the role of physiologic sex hormone levels and their variation during aging in anticancer immunity, studying the immune system of individuals with pathologic hormone levels or genetic mutations blocking or diminishing male sex differentiation of individuals with XY chromosomes and with or without functioning testicles (e.g., SRY, SOX 9, and AR mutations) could help dissecting the effect of sex hormones from that of sex chromosomes (92, 93).

Furthermore, elucidating the relationship between sex hormones, obesity, the gut microbiome, and immune responses in men and women could improve our understanding of resistance mechanisms to immune checkpoint inhibitors and better select patients who might benefit from these costly therapies.

While immune signals appear to play a role in the reactivation of disseminated tumor cells (DTCs) surviving in a "dormant" state in distant organs, the influence of sex hormones in this context is, however, currently unknown (94). Because hormone-responsive tumors such as ER-positive breast cancer and prostate cancer can relapse after years or even decades of apparent remission, investigation of a possible association between changes of sex hormone levels during aging or pharmacologic treatment and reawakening of DTCs is of great clinical interest (Fig. 1).

Various topics such as the impact of pregnancy on cancer relapse are still a matter of debate with controversial findings (95). There is an unmet need to thoroughly characterize the immunologic changes that occur during pregnancy and systematically collect data on pregnancy-associated cancers. Evidence-based recommendations regarding pregnancy are required to appropriately counsel the increasing population of cancer survivors in child-bearing age.

Finally, we need to revisit clinical trial design in immuno-oncology. The trend to empirically combine different immunotherapy approaches with or without standard therapies is increasingly questionable. This approach should be replaced by rational combination strategies based on a better understanding of the mechanism of action and the effects of sex chromosomes and hormones on immune responses. Also, the reporting of trial results should contain subgroup analyses according to sex and discuss whether the study was sufficiently powered to detect potentially relevant sex differences, the plausibility of the findings, as well as their biological basis. A close collaboration between different institutions and data sharing could help advance the field of immuno-oncology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. Clocchiatti A, Cora E, Zhang Y, Dotto GP. Sexual dimorphism in cancer. Nat Rev Cancer 2016;16:330–9.
3. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56–61.
4. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007;450:903–7.
5. Chapman JR, Webster AC, Wong G. Cancer in the transplant recipient. Cold Spring Harb Perspect Med 2013;3:a015677.
6. Yarchoan R, Uldrick TS. HIV-associated cancers and related diseases. N Engl J Med 2018;378:1029–41.
7. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017;541:321–30.
8. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016;16:626–38.
9. Pierdominici M, Maselli A, Colasanti T, Giammarioli AM, Delunardo F, Vacirca D, et al. Estrogen receptor profiles in human peripheral blood lymphocytes. Immunol Lett 2010;132:79–85.
10. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. Cell Immunol 2015;294:63–9.
11. Mantalaris A, Panoskaltsis N, Sakai Y, Bourne P, Chang C, Messing EM, et al. Localization of androgen receptor expression in human bone marrow. J Pathol 2001;193:361–6.
12. Arruvito L, Giulianelli S, Flores AC, Paladino N, Barboza M, Lanari C, et al. NK cells expressing a progesterone receptor are susceptible to progesterone-induced apoptosis. J Immunol 2008;180:5746–53.
13. Dosiou C, Hamilton AE, Pang Y, Overgaard MT, Tulac S, Dong J, et al. Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. J Endocrinol 2008;196:67–77.
14. Hannah MF, Bajic VB, Klein SL. Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. Brain Behav Immun 2008;22:503–16.
15. Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007;28:521–74.
16. Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thiebaut R, et al. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci U S A 2014;111:869–74.
17. Kissick HT, Sanda MG, Dunn LK, Pellegrini KL, On ST, Noel JK, et al. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. Proc Natl Acad Sci U S A 2014;111:9887–92.
18. Kovats S, Carreras E. Regulation of dendritic cell differentiation and function by estrogen receptor ligands. Cell Immunol 2008;252:81–90.
19. Castelo-Branco C, Soveral I. The immune system and aging: a review. Gynecol Endocrinol 2014;30:16–22.
20. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab 2005;90:3847–53.

21. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR, Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2001;86:724–31.
22. Horstman AM, Dillon EL, Urban RJ, Sheffield-Moore M. The role of androgens and estrogens on healthy aging and longevity. J Gerontol A Biol Sci Med Sci 2012;67:1140–52.
23. Wang Y, Gay GD, Botelho JC, Caudill SP, Vesper HW. Total testosterone quantitative measurement in serum by LC-MS/MS. Clin Chim Acta 2014; 436:263–7.
24. Burger HG. Androgen production in women. Fertil Steril 2002;77:S3–5.
25. Vermeulen A, Oddens BJ. Declining androgens with age: an overview. New York, NY: Parthenon Publishing;1996. p. 3–14.
26. Merlotti D, Gennari L, Stolakis K, Nuti R. Aromatase activity and bone loss in men. J Osteoporos 2011;2011:230671.
27. Orwoll E, Lambert LC, Marshall LM, Phipps K, Blank J, Barrett-Connor E, et al. Testosterone and estradiol among older men. J Clin Endocrinol Metab 2006;91:1336–44.
28. Jasuja GK, Travison TG, Davda M, Murabito JM, Basaria S, Zhang A, et al. Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. J Gerontol A Biol Sci Med Sci 2013;68:733–40.
29. Yialamas MA, Hayes FJ. Androgens and the ageing male and female. Best Pract Res Clin Endocrinol Metab 2003;17:223–36.
30. Yan J, Greer JM, Hull R, O'Sullivan JD, Henderson RD, Read SJ, et al. The effect of ageing on human lymphocyte subsets: comparison of males and females. Immun Ageing 2010;7:4.
31. Hirokawa K, Utsuyama M, Hayashi Y, Kitagawa M, Makinodan T, Fulop T. Slower immune system aging in women versus men in the Japanese population. Immun Ageing 2013;10:19.
32. Gubbels Bupp MR, Potluri T, Fink AL, Klein SL. The confluence of sex hormones and aging on immunity. Front Immunol 2018;9:1269.
33. Campisi J. Cellular senescence: putting the paradoxes in perspective. Curr Opin Genet Dev 2011;21:107–12.
34. Clocchiatti A, Ghosh S, Procopio MG, Mazzeo L, Bordignon P, Ostano P, et al. Androgen receptor functions as transcriptional repressor of cancer-associated fibroblast activation. J Clin Invest 2018;128:5531–48.
35. Beral V; Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 2003;362: 419–27.
36. Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 1995;85:304–13.
37. Lacey JV Jr, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, et al. Menopausal hormone replacement therapy and risk of ovarian cancer. JAMA 2002;288:334–41.
38. Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat 2008;107:103–11.
39. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701–12.
40. Porter VR, Greendale GA, Schocken M, Zhu X, Effros RB. Immune effects of hormone replacement therapy in post-menopausal women. Exp Gerontol 2001;36:311–26.
41. Ghosh M, Rodriguez-Garcia M, Wira CR. The immune system in menopause: pros and cons of hormone therapy. J Steroid Biochem Mol Biol 2014;142:171–5.
42. Morch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard O. Contemporary hormonal contraception and the risk of breast cancer. N Engl J Med 2017;377:2228–39.
43. Auerbach L, Hafner T, Huber JC, Panzer S. Influence of low-dose oral contraception on peripheral blood lymphocyte subsets at particular phases of the hormonal cycle. Fertil Steril 2002;78:83–9.
44. Madeleine MM, Daling JR, Schwartz SM, Shera K, McKnight B, Carter JJ, et al. Human papillomavirus and long-term oral contraceptive use increase the risk of adenocarcinoma in situ of the cervix. Cancer Epidemiol Biomarkers Prev 2001;10:171–7.
45. Ghanem KG, Datta SD, Unger ER, Hagensee M, Shlay JC, Kerndt P, et al. The association of current hormonal contraceptive use with type-specific HPV detection. Sex Transm Infect 2011;87:385–8.
46. Collaborative Group on Epidemiological Studies of Ovarian Cancer, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet 2008;371:303–14.
47. Iodice S, Barile M, Rotmensz N, Feroce I, Bonanni B, Radice P, et al. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. Eur J Cancer 2010;46:2275–84.
48. Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, et al. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomarkers Prev 2013;22:1931–43.
49. Calof OM, Singh AB, Lee ML, Kenny AM, Urban RJ, Tenover JL, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. J Gerontol A Biol Sci Med Sci 2005;60:1451–7.
50. Feneley MR, Carruthers M. Is testosterone treatment good for the prostate? Study of safety during long-term treatment. J Sex Med 2012;9:2138–49.
51. Eisenberg ML, Li S, Betts P, Herder D, Lamb DJ, Lipshultz LI. Testosterone therapy and cancer risk. BJU Int 2015;115:317–21.
52. Khera M, Crawford D, Morales A, Salonia A, Morgentaler A. A new era of testosterone and prostate cancer: from physiology to clinical implications. Eur Urol 2014;65:115–23.
53. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
54. Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Estrogen-mediated immunomodulation involves reduced activation of effector T cells, potentiation of Treg cells, and enhanced expression of the PD-1 costimulatory pathway. J Neurosci Res 2006;84:370–8.
55. Wang C, Dehghani B, Li Y, Kaler LJ, Vandenbark AA, Offner H. Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death 1. Immunology 2009;126:329–35.
56. Nosrati A, Tsai KK, Goldinger SM, Tumeh P, Grimes B, Loo K, et al. Evaluation of clinicopathological factors in PD-1 response: derivation and validation of a prediction scale for response to PD-1 monotherapy. Br J Cancer 2017;116:1141–7.
57. Botticelli A, Onesti CE, Zizzari I, Cerbelli B, Sciattella P, Occhipinti M, et al. The sexist behaviour of immune checkpoint inhibitors in cancer therapy? Oncotarget 2017;8:99336–46.
58. Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. Lancet Oncol 2018;19:737–46.
59. Loo K, Tsai KK, Mahuron K, Liu J, Pauli ML, Sandoval PM, et al. Partially exhausted tumor-infiltrating lymphocytes predict response to combination immunotherapy. JCI Insight 2017;2:93433.
60. Maleki Vareki S, Garrigos C, Duran I. Biomarkers of response to PD-1/PD-L1 inhibition. Crit Rev Oncol Hematol 2017;116:116–24.
61. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2017;359:97–103.
62. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91–7.
63. Org E, Mehrabian M, Parks BW, Shipkova P, Liu X, Drake TA, et al. Sex differences and hormonal effects on gut microbiota composition in mice. Gut Microbes 2016;7:313–22.
64. Haro C, Rangel-Zuniga OA, Alcala-Diaz JF, Gomez-Delgado F, Perez-Martinez P, Delgado-Lista J, et al. Intestinal microbiota is influenced by gender and body mass index. PLoS One 2016;11:e0154090.
65. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 2013;339:1084–8.
66. Zitvogel L, Galluzzi L, Viaud S, Vetizou M, Daillere R, Merad M, et al. Cancer and the gut microbiota: an unexpected link. Sci Transl Med 2015;7:271ps1.
67. McQuade JL, Daniel CR, Hess KR, Mak C, Wang DY, Rai RR, et al. Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. Lancet Oncol 2018;19:310–22.

68. Pan Y, Zheng D, Li Y, Cai X, Zheng Z, Jin Y, et al. Unique distribution of programmed death ligand 1 (PD-L1) expression in East Asian non-small cell lung cancer. J Thorac Dis 2017;9:2579–86.
69. Wu S, Shi X, Sun J, Liu Y, Luo Y, Liang Z, et al. The significance of programmed cell death ligand 1 expression in resected lung adenocarcinoma. Oncotarget 2017;8:16421–9.
70. Cogdill AP, Andrews MC, Wargo JA. Hallmarks of response to immune checkpoint blockade. Br J Cancer 2017;117:1–7.
71. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Min L, Krop IE, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens: a systematic review and meta-analysis. JAMA Oncol 2018;4:173–82.
72. Jaini R, Loya MG, Eng C. Immunotherapeutic target expression on breast tumors can be amplified by hormone receptor antagonism: a novel strategy for enhancing efficacy of targeted immunotherapy. Oncotarget 2017;8: 32536–49.
73. Corriden R, Hollands A, Olson J, Derieux J, Lopez J, Chang JT, et al. Tamoxifen augments the innate immune function of neutrophils through modulation of intracellular ceramide. Nat Commun 2015;6:8369.
74. Svoronos N, Perales-Puchalt A, Allegrezza MJ, Rutkowski MR, Payne KK, Tesone AJ, et al. Tumor cell-independent estrogen signaling drives disease progression through mobilization of myeloid-derived suppressor cells. Cancer Discov 2017;7:72–85.
75. Generali D, Bates G, Berruti A, Brizzi MP, Campo L, Bonardi S, et al. Immunomodulation of FOXP3+ regulatory T cells by the aromatase inhibitor letrozole in breast cancer patients. Clin Cancer Res 2009;15: 1046–51.
76. Williams KM, Lucas PJ, Bare CV, Wang J, Chu YW, Tayler E, et al. CCL25 increases thymopoiesis after androgen withdrawal. Blood 2008;112: 3255–63.
77. Olsen NJ, Viselli SM, Fan J, Kovacs WJ. Androgens accelerate thymocyte apoptosis. Endocrinology 1998;139:748–52.
78. Musabak U, Bolu E, Ozata M, Oktenli C, Sengul A, Inal A, et al. Gonadotropin treatment restores in vitro interleukin-1beta and tumour necrosis factor-alpha production by stimulated peripheral blood mononuclear cells from patients with idiopathic hypogonadotropic hypogonadism. Clin Exp Immunol 2003;132:265–70.
79. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab 2004;89: 3313–8.
80. Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, Saad F. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. Clin Endocrinol 2010;73:602–12.
81. Morse MD, McNeel DG. Prostate cancer patients on androgen deprivation therapy develop persistent changes in adaptive immune responses. Hum Immunol 2010;71:496–504.
82. Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci U S A 2001;98: 14565–70.
83. Escamilla J, Schokrpur S, Liu C, Priceman SJ, Moughon D, Jiang Z, et al. CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy. Cancer Res 2015; 75:950–62.
84. Ardiani A, Gameiro SR, Kwilas AR, Donahue RN, Hodge JW. Androgen deprivation therapy sensitizes prostate cancer cells to T-cell killing through androgen receptor dependent modulation of the apoptotic pathway. Oncotarget 2014;5:9335–48.
85. Olson BM, Gamat M, Seliski J, Sawicki T, Jeffery J, Ellis L, et al. Prostate cancer cells express more androgen receptor (AR) following androgen deprivation, improving recognition by AR-specific T cells. Cancer Immunol Res 2017;5:1074–85.
86. McNeel DG, Smith HA, Eickhoff JC, Lang JM, Staab MJ, Wilding G, et al. Phase I trial of tremelimumab in combination with short-term androgen deprivation in patients with PSA-recurrent prostate cancer. Cancer Immunol Immunother 2012;61:1137–47.
87. Small EJ, Lance RS, Gardner TA, Karsh LI, Fong L, McCoy C, et al. A Randomized phase II trial of Sipuleucel-T with concurrent versus sequential abiraterone acetate plus prednisone in metastatic castration-resistant prostate cancer. Clin Cancer Res 2015;21:3862–9.
88. Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, et al. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res 2008;14:4526–31.
89. Pu Y, Xu M, Liang Y, Yang K, Guo Y, Yang X, et al. Androgen receptor antagonists compromise T cell response against prostate cancer leading to early tumor relapse. Sci Transl Med 2016;8:333ra47.
90. Petrylak DPD CG, Pieczonka CM, Corman JM, Garcia JA, Dunshee C, van Mouwerik T, et al. Overall survival and immune responses with sipuleucel-T and enzalutamide: STRIDE study. J Clin Oncol 2018;36: 246–6.
91. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 2010;10: 594–604.
92. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, MacDougall J. Androgen insensitivity syndrome. Lancet 2012;380: 1419–28.
93. Harley VR, Clarkson MJ, Argentaro A. The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. Endocr Rev 2003;24:466–87.
94. Mohme M, Riethdorf S, Pantel K. Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. Nat Rev Clin Oncol 2017;14:155–67.
95. Todd SP, Driscoll MS. Prognosis for women diagnosed with melanoma during, before, or after pregnancy: weighing the evidence. Int J Womens Dermatol 2017;3:26–9.
96. Friedrich N, Volzke H, Rosskopf D, Steveling A, Krebs A, Nauck M, et al. Reference ranges for serum dehydroepiandrosterone sulfate and testosterone in adult men. J Androl 2008;29:610–7.
97. Stanczyk FZ, Clarke NJ. Measurement of estradiol–challenges ahead. J Clin Endocrinol Metab 2014;99:56–8.
98. Ray JA, Kushnir MM, Bunker A, Rockwood AL, Meikle AW. Direct measurement of free estradiol in human serum by equilibrium dialysis-liquid chromatography-tandem mass spectrometry and reference intervals of free estradiol in women. Clin Chim Acta 2012;413: 1008–14.