Title: Sex Hormones and Anticancer Immunity.

Authors: Özdemir BC, Dotto GP

Journal: Clinical cancer research : an official journal of the American Association for Cancer Research

Year: 2019 Aug 1

Issue: 25

Volume: 15

Pages: 4603-4610

DOI: 10.1158/1078-0432.CCR-19-0137

Sex hormones and anticancer immunity

Berna C. Özdemir MD PhD[1, 2],Gian-Paolo Dotto MD PhD [2 ,3, 4]

1 Department of Oncology, Lausanne University Hospital, Lausanne, CH-1011, Switzerland

2 International Cancer Prevention Institute, Epalinges 1066, Switzerland

3 Department of Biochemistry, University of Lausanne, Epalinges, CH-1066, Switzerland

4 Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, MA 02129, USA

Corresponding author:

Özdemir, B.C (berna.ozdemir@chuv.ch)

The authors have no conflict of interest to declare.

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 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 immunooncology 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 postmenopausal hormone therapies and oral contraception. We will review emerging data on sex differences in PD-L1 expression and the efficacy of immune checkpoint inhibitors 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. Lastly, we will point to areas of future research.

Keywords

Sex differences

Gender differences

Cancer

Immunity

Immunotherapy

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. In contrast, in "immune-excluded” tumors, which also contain abundant immune cells, the development of an effective antitumor immune response is blocked by the retention of immune cells in the tumor stroma , while tumors characterized by a paucity of T cells (“immune desert”) do not activate the innate immune system in the first place, corresponding to a state of immune exclusion [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 non-reproductive 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 (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 paper, for a recent review on the hormonal effects on immune cells see [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 cancer patients is currently unknown. Additionally, an essential question to be addressed is how the throughout lifetime changing levels of estrogens and androgens in humans affect various immune cell types and ultimately the clinical behavior and treatment outcome of various cancers.

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. Specifically, aging exerts a significant impact on estrogen and androgen levels and signalling, differently between males and females and is itself

controlled by these hormones. Aging is associated with a rapid decrease in estrogen levels in women and a rather progressive decline in androgen concentrations in men [19]. Women synthesize androgens and their precursors (e.g. DHEAs) in the adrenal glands and ovaries [20] with premenopausal total testosterone levels corresponding to one-twentieth of those found in adult men [21] [22]. In the absence of assays allowing accurate measurement of the low levels of testosterone in women, the occurrence of androgen deficiency in post-menopausal women is debated [23]. In men, testosterone is reduced to dihydrotestosterone (DHT), which binds more avidly to the AR and is converted into estradiol by the cytochrome P450 aromatase present in adipose tissue, skin, bone and other organs [24]. Some studies [25], but not others [26], 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 [25]. Additionally, an individual's true sex hormone status during lifetime is most likely determined by the combination of serum estradiol, testosterone and dihydrotestosterone levels as well as body mass index (BMI) and sex hormone receptor activity [27].

The accelerated rate of 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 to aging women [28, 29]. In women, menopause is associated with an increase in pro-inflammatory IL-1β, IL6 and TNFα levels and a reduction in the anti-inflammatory IFNγ levels. Monocytes and NK cells of aged women exhibit a pro-inflammatory phenotype and more robust cytotoxic activity, respectively, compared to those of aged men. For a recent comprehensive review on the effect of sex hormones on aging and immunity see [30].

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 cancer [31]. Although not reported, it is likely that the physiological decline in sex hormones during aging plays a role in cellular senescence.

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 should be further investigated. In postmenopausal women, hormone replacement therapies (HRT) are associated with an increased cancer risk of hormone-responsive tissues such as breast [32], endometrium [33] and ovaries [34]. The response of different tissues and cell types to HRT might depend on the presence of concomitant risk factors such as obesity and the individual genetic background. It was reported that HRT partially reverses the impact of aging on immunity by increasing B and T cell counts [35], and by decreasing levels of pro-inflammatory cytokines - TNFα and IL-6- in postmenopausal women [36].

Likewise, various forms of hormonal contraception (HC), mostly administered as oral estrogen-progestin combinations, correlate with increased breast cancer risk (analysis of data from 1.8 million women) [37]. This occurs despite increased numbers of B and T cells [38], 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 HC might also alter its impact on immune responses. Long-term oral HC is also correlated with increased risk of adenocarcinoma in situ of the cervix [39]. While infection with human papillomavirus (HPV) and immunosuppression are established causes of cervix cancer, reports on the association between long-term HC use and impaired virus clearance resulting in persistent HPV infections are inconsistent. It is possible that only some oncogenic HPV types, such as HPV16, are associated with HC exposure [40]. Given that most women will be infected with HPV during their lifetime, a possible negative effect of HC on immune responses against these viruses needs to be evaluated.

In contrast, HC use is associated with a significant reduction of ovarian cancer risk (data from forty-five epidemiological studies) [41] even in BRCA 1 or 2 gene mutation carriers [42]. Also, a decreased risk for colorectal and endometrial cancer has been found in HC users [43]. The mechanisms behind these opposing effects of postmenopausal HRT and HC on ovarian and endometrial cancer risk are currently unclear and it needs to be determined how the interference with physiological 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.

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 CTLA-4, which is constitutively expressed in regulatory T cells and upregulated upon activation of naïve T

cells; PD-1, which is found in T cells, B cells and natural killer (NK) cells; and the PD-L1, a PD-1 ligand expressed in antigen presenting cells and cancer cells [44]. Some animal studies and emerging clinical evidence suggest a role for estrogens in upregulation of PD-1 and PD-L1 expression [45, 46], and for sex differences in the response to immune checkpoint inhibitors [47-49].

Female sex has been suggested as a negative predictive factor for response of melanoma patients to anti-PD1-therapy [47]. 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 [50], while an hormone-mediated mechanism might also be important. However, in absence of pre-planned 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, beyond high PD-L1 expression, high tumor mutational burden or the presence of tumor infiltrating lymphocytes (TILs) [51], are lacking. The gut microbiome is emerging as a modulator of response to immune checkpoint inhibitors. In a recent study of melanoma patients undergoing anti-PD1 therapy, significant differences were found in the diversity and composition of the gut microbiome of responders compared to non-responders. Patients with the most diverse microbiome were more likely to respond to immunotherapy, while antibiotic therapy was predictive of resistance to anti-PD1 blockade. Fecal transplants from responders to germ-free or antibiotics treated mice resulted in increased anti-tumor immunity with reduced tumor growth [52] [53]. 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 [54, 55].

Although very likely, the crosstalk 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 [56]. 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 cancer patients 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 [57] [58]. Since in current clinical practice PD-L1 positivity is mostly correlated with poor prognosis but predictive of response to immune checkpoint inhibitors [51], 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-CTLA4 or anti-PD1 therapies compared to women [49]. 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 (Figure 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 [59]. 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 [60].

Sex hormone deprivation therapies as co-adjuvants 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. Since these therapies inhibit cancer cells, it is difficult to evaluate their immunomodulatory effects.

Anti-estrogen 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 [61]. This upregulation in antigen expression is correlated with increased anticancer immunity given that tumor-bearing mice respond to treatment with antigen-specific lymphocyte transfer [61]. Tamoxifen stimulates neutrophil activity in vitro and in vivo through modulation of sphingolipid biosynthesis [62] and was shown to diminish the number of immunosuppressive myeloid derived suppressor cells (MDSCs) and increase the population of effector and cytotoxic T cells that infiltrated the tumor in a mouse model of ERα negative ovarian cancer [63].

The aromatase inhibitor letrozole significantly reduces the number of Tregs in human breast cancer tissue, which is correlated with therapy response [64].

These results suggest a potential role for anti-estrogen 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 [65, 66]. Immune cells isolated from men with androgen deficiencies produce more pro-inflammatory cytokines such as IL-1β, IL-2 and TNFα when stimulated with lipopolysaccharides (LPS) [67], which is reverted upon androgen replacement [67] [68] [69]. 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 1-24 months [70]. Histologically, ADT is associated with a strong T cell and macrophage infiltration into the prostate after 1 week of treatment [71, 72]. Several studies demonstrated that ADT enhances susceptibility of AR-overexpressing prostate cancer cells to immune-mediated T cell killing through improved immune recognition [73] [74]. Emerging clinical data also reveals that ADT enhances the efficacy of various immunotherapies including immune checkpoint blockade [75] and cancer vaccines such as spileucel T [76] and Prostvac [77].

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 prostate cancer patients to immune checkpoint inhibitors (Table 2).

Although the optimal timing and duration of such therapies remain to be determined, animal experiments [78] and data from a phase II clinical trial [77] suggest

that sequential therapy with administration of immunotherapy before AR antagonists rather than after or concomitant to ADT could improve therapeutic responses.

Conclusions and areas of future research

Despite the impressive achievements in the field of immunooncology, 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 which 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 [79]. In addition to investigating the role of physiological sex hormone levels and their variation during aging in anticancer immunity, studying the immune system of individuals with pathological 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 [80, 81].

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 [82]. Since 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 pharmacological treatment and reawakening of DTCs is of great clinical interest (Figure 1).

Various topics such as the impact of pregnancy on cancer relapse are still a matter of debate with controversial findings [83]. There is an unmet need to thoroughly characterize the immunological 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.

Lastly, we need to revisit clinical trial design in immunooncology. 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 immunooncology.

Acknowledgments

We thank Dr Andrea Clocchiatti for critical reading of the manuscript and useful comments. This work was supported by grants from the National Institutes of Health (R01AR039190; R01AR064786; the content not necessarily representing the official

views of NIH), the Swiss National Science Foundation (310030_156191/1) and European Research Council (26075083) to GPD.

References

1. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA Cancer J Clin, 2018. **68**(6): p. 394-424.
2. Clocchiatti, A., et al., *Sexual dimorphism in cancer.* Nat Rev Cancer, 2016. **16**(5): p. 330-9.
3. Sharma, P. and J.P. Allison, *The future of immune checkpoint therapy.* Science, 2015. **348**(6230): p. 56-61.
4. Koebel, C.M., et al., *Adaptive immunity maintains occult cancer in an equilibrium state.* Nature, 2007. **450**(7171): p. 903-7.
5. Chapman, J.R., A.C. Webster, and G. Wong, *Cancer in the transplant recipient.* Cold Spring Harb Perspect Med, 2013. **3**(7).
6. Yarchoan, R. and T.S. Uldrick, *HIV-Associated Cancers and Related Diseases.* N Engl J Med, 2018. **378**(11): p. 1029-1041.
7. Chen, D.S. and I. Mellman, *Elements of cancer immunity and the cancer-immune set point.* Nature, 2017. **541**(7637): p. 321-330.
8. Klein, S.L. and K.L. Flanagan, *Sex differences in immune responses.* Nat Rev Immunol, 2016. **16**(10): p. 626-38.
9. Pierdominici, M., et al., *Estrogen receptor profiles in human peripheral blood lymphocytes.* Immunol Lett, 2010. **132**(1-2): p. 79-85.
10. Kovats, S., *Estrogen receptors regulate innate immune cells and signaling pathways.* Cell Immunol, 2015. **294**(2): p. 63-9.
11. Mantalaris, A., et al., *Localization of androgen receptor expression in human bone marrow.* J Pathol, 2001. **193**(3): p. 361-6.
12. Arruvito, L., et al., *NK cells expressing a progesterone receptor are susceptible to progesterone-induced apoptosis.* J Immunol, 2008. **180**(8): p. 5746-53.
13. Dosiou, C., 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**(1): p. 67-77.
14. Hannah, M.F., V.B. Bajic, and S.L. Klein, *Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats.* Brain Behav Immun, 2008. **22**(4): p. 503-16.
15. Straub, R.H., *The complex role of estrogens in inflammation.* Endocr Rev, 2007. **28**(5): p. 521-74.
16. Furman, D., 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**(2): p. 869-74.
17. Kissick, H.T., et al., *Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation.* Proc Natl Acad Sci U S A, 2014. **111**(27): p. 9887-92.

18. Kovats, S. and E. Carreras, *Regulation of dendritic cell differentiation and function by estrogen receptor ligands.* Cell Immunol, 2008. **252**(1-2): p. 81-90.
19. Castelo-Branco, C. and I. Soveral, *The immune system and aging: a review.* Gynecol Endocrinol, 2014. **30**(1): p. 16-22.
20. Burger, H.G., *Androgen production in women.* Fertil Steril, 2002. **77 Suppl 4**: p. S3-5.
21. Davison, S.L., et al., *Androgen levels in adult females: changes with age, menopause, and oophorectomy.* J Clin Endocrinol Metab, 2005. **90**(7): p. 3847-53.
22. Vermeulen, A.O., BJ; , *Declining androgens with age: an overview.* New York: Parthenon Publishing, 1996: p. 3-14.
23. Morley, J.E. and H.M. Perry, 3rd, *Androgens and women at the menopause and beyond.* J Gerontol A Biol Sci Med Sci, 2003. **58**(5): p. M409-16.
24. Merlotti, D., et al., *Aromatase activity and bone loss in men.* J Osteoporos, 2011. **2011**: p. 230671.
25. Orwoll, E., et al., *Testosterone and estradiol among older men.* J Clin Endocrinol Metab, 2006. **91**(4): p. 1336-44.
26. Jasuja, G.K., 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**(6): p. 733-40.
27. Yialamas, M.A. and F.J. Hayes, *Androgens and the ageing male and female.* Best Pract Res Clin Endocrinol Metab, 2003. **17**(2): p. 223-36.
28. Yan, J., et al., *The effect of ageing on human lymphocyte subsets: comparison of males and females.* Immun Ageing, 2010. **7**: p. 4.
29. Hirokawa, K., et al., *Slower immune system aging in women versus men in the Japanese population.* Immun Ageing, 2013. **10**(1): p. 19.
30. Gubbels Bupp, M.R., et al., *The Confluence of Sex Hormones and Aging on Immunity.* Front Immunol, 2018. **9**: p. 1269.
31. Campisi, J., *Cellular senescence: putting the paradoxes in perspective.* Curr Opin Genet Dev, 2011. **21**(1): p. 107-12.
32. Beral, V. and C. Million Women Study, *Breast cancer and hormone-replacement therapy in the Million Women Study.* Lancet, 2003. **362**(9382): p. 419-27.
33. Grady, D., et al., *Hormone replacement therapy and endometrial cancer risk: a meta-analysis.* Obstet Gynecol, 1995. **85**(2): p. 304-13.
34. Lacey, J.V., Jr., et al., *Menopausal hormone replacement therapy and risk of ovarian cancer.* JAMA, 2002. **288**(3): p. 334-41.
35. Porter, V.R., et al., *Immune effects of hormone replacement therapy in post-menopausal women.* Exp Gerontol, 2001. **36**(2): p. 311-26.
36. Ghosh, M., M. Rodriguez-Garcia, and C.R. Wira, *The immune system in menopause: pros and cons of hormone therapy.* J Steroid Biochem Mol Biol, 2014. **142**: p. 171-5.
37. Morch, L.S., et al., *Contemporary Hormonal Contraception and the Risk of Breast Cancer.* N Engl J Med, 2017. **377**(23): p. 2228-2239.
38. Auerbach, L., et al., *Influence of low-dose oral contraception on peripheral blood lymphocyte subsets at particular phases of the hormonal cycle.* Fertil Steril, 2002. **78**(1): p. 83-9.
39. Madeleine, M.M., 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**(3): p. 171-7.

40. Ghanem, K.G., et al., *The association of current hormonal contraceptive use with type-specific HPV detection.* Sex Transm Infect, 2011. **87**(5): p. 385-8.
41. Collaborative Group on Epidemiological Studies of Ovarian, C., et al., *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**(9609): p. 303-14.
42. Iodice, S., et al., *Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis.* Eur J Cancer, 2010. **46**(12): p. 2275-84.
43. Gierisch, J.M., et al., *Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review.* Cancer Epidemiol Biomarkers Prev, 2013. **22**(11): p. 1931-43.
44. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy.* Nat Rev Cancer, 2012. **12**(4): p. 252-64.
45. Polanczyk, M.J., et al., *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**(2): p. 370-8.
46. Wang, C., et al., *Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death 1.* Immunology, 2009. **126**(3): p. 329-35.
47. Nosrati, A., 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**(9): p. 1141-1147.
48. Botticelli, A., et al., *The sexist behaviour of immune checkpoint inhibitors in cancer therapy?* Oncotarget, 2017. **8**(59): p. 99336-99346.
49. Conforti, F., et al., *Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis.* Lancet Oncol, 2018.
50. Loo, K., et al., *Partially exhausted tumor-infiltrating lymphocytes predict response to combination immunotherapy.* JCI Insight, 2017. **2**(14).
51. Maleki Vareki, S., C. Garrigos, and I. Duran, *Biomarkers of response to PD-1/PD-L1 inhibition.* Crit Rev Oncol Hematol, 2017. **116**: p. 116-124.
52. Gopalakrishnan, V., et al., *Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.* Science, 2017.
53. Routy, B., et al., *Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors.* Science, 2017.
54. Org, E., et al., *Sex differences and hormonal effects on gut microbiota composition in mice.* Gut Microbes, 2016. **7**(4): p. 313-322.
55. Haro, C., et al., *Intestinal Microbiota Is Influenced by Gender and Body Mass Index.* PLoS One, 2016. **11**(5): p. e0154090.
56. McQuade, J.L., 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**(3): p. 310-322.
57. Pan, 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**(8): p. 2579-2586.
58. Wu, S., et al., *The significance of programmed cell death ligand 1 expression in resected lung adenocarcinoma.* Oncotarget, 2017. **8**(10): p. 16421-16429.

59. Cogdill, A.P., M.C. Andrews, and J.A. Wargo, *Hallmarks of response to immune checkpoint blockade.* Br J Cancer, 2017. **117**(1): p. 1-7.
60. Barroso-Sousa, R., 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**(2): p. 173-182.
61. Jaini, R., M.G. Loya, and C. Eng, *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**(20): p. 32536-32549.
62. Corriden, R., et al., *Tamoxifen augments the innate immune function of neutrophils through modulation of intracellular ceramide.* Nat Commun, 2015. **6**: p. 8369.
63. Svoronos, N., et al., *Tumor Cell-Independent Estrogen Signaling Drives Disease Progression through Mobilization of Myeloid-Derived Suppressor Cells.* Cancer Discov, 2017. **7**(1): p. 72-85.
64. Generali, D., et al., *Immunomodulation of FOXP3+ regulatory T cells by the aromatase inhibitor letrozole in breast cancer patients.* Clin Cancer Res, 2009. **15**(3): p. 1046-51.
65. Williams, K.M., et al., *CCL25 increases thymopoiesis after androgen withdrawal.* Blood, 2008. **112**(8): p. 3255-63.
66. Olsen, N.J., et al., *Androgens accelerate thymocyte apoptosis.* Endocrinology, 1998. **139**(2): p. 748-52.
67. Musabak, U., 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**(2): p. 265-70.
68. Malkin, C.J., et al., *The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men.* J Clin Endocrinol Metab, 2004. **89**(7): p. 3313-8.
69. Kalinchenko, S.Y., et al., *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 (Oxf), 2010. **73**(5): p. 602-12.
70. Morse, M.D. and D.G. McNeel, *Prostate cancer patients on androgen deprivation therapy develop persistent changes in adaptive immune responses.* Hum Immunol, 2010. **71**(5): p. 496-504.
71. Mercader, M., 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**(25): p. 14565-70.
72. Escamilla, J., et al., *CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy.* Cancer Res, 2015. **75**(6): p. 950-62.
73. Ardiani, A., et al., *Androgen deprivation therapy sensitizes prostate cancer cells to T-cell killing through androgen receptor dependent modulation of the apoptotic pathway.* Oncotarget, 2014. **5**(19): p. 9335-48.
74. Olson, B.M., et al., *Prostate Cancer Cells Express More Androgen Receptor (AR) Following Androgen Deprivation, Improving Recognition by AR-Specific T Cells.* Cancer Immunol Res, 2017.

75. McNeel, D.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**(7): p. 1137-47.
76. Small, E.J., 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**(17): p. 3862-9.
77. Madan, R.A., 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**(14): p. 4526-31.
78. Pu, Y., et al., *Androgen receptor antagonists compromise T cell response against prostate cancer leading to early tumor relapse.* Sci Transl Med, 2016. **8**(333): p. 333ra47.
79. Libert, C., L. Dejager, and I. Pinheiro, *The X chromosome in immune functions: when a chromosome makes the difference.* Nat Rev Immunol, 2010. **10**(8): p. 594-604.
80. Hughes, I.A., et al., *Androgen insensitivity syndrome.* Lancet, 2012. **380**(9851): p. 1419-28.
81. Harley, V.R., M.J. Clarkson, and A. Argentaro, *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**(4): p. 466-87.
82. Mohme, M., S. Riethdorf, and K. Pantel, *Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape.* Nat Rev Clin Oncol, 2017. **14**(3): p. 155-167.
83. Todd, S.P. and M.S. Driscoll, *Prognosis for women diagnosed with melanoma during, before, or after pregnancy: Weighing the evidence.* Int J Womens Dermatol, 2017. **3**(1): p. 26-29.

	Age	Men	Women	Ref
Mean total Testosterone (ng/dl)	25-54	469-553	23-45	[1-3]
	> 55	469-475	19-20	
Mean free Testosterone (ng/dl)	25-54	9-12	0.3-0.7	[1-3]
	>55	7-8.3	0.3	
Mean DHEAS (µg/dl)	25-54	151-286	126-276	[2, 3]
	>55	114-137	65-87	
Mean Estradiol (pg/ml)	25-54	25.1-25.7	30-800	[4, 5]
	>55 /postmenopausal	25.7-29.7	<20	
Mean free Estradiol (pg/ml)	25-54	0.54-0.56	2.4-3.1	[4, 6]
	>55/ postmenopausal	0.46-0.53	<0.5	

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

References

1. Vermeulen, A.O., BJ; , *Declining androgens with age: an overview.* New York: Parthenon Publishing, 1996: p. 3-14.
2. Davison, S.L., et al., *Androgen levels in adult females: changes with age, menopause, and oophorectomy.* J Clin Endocrinol Metab, 2005. **90**(7): p. 3847-53.
3. Friedrich, N., et al., *Reference ranges for serum dehydroepiandrosterone sulfate and testosterone in adult men.* J Androl, 2008. **29**(6): p. 610-7.
4. Jasuja, G.K., 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**(6): p. 733-40.
5. Stanczyk, F.Z. and N.J. Clarke, *Measurement of estradiol--challenges ahead.* J Clin Endocrinol Metab, 2014. **99**(1): p. 56-8.
6. Ray, J.A., et al., *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**(11-12): p. 1008-14.

Indication	Drugs	Phase	Study ID	Number of participants, Primary endpoints	Study completion date
ER+ HER2- BC	Exemestane + Tremelimumab Exemestane + Durvalumab	Phase 1 Phase 2	NCT02997995 ULTIMATE	N=240, pathological CR	Sep 2020
ER+ BC	Letrozole + Pembrolizumab +Palbociclib	Phase 2	NCT02778685	N= 22, ORR	Sep 2018
HR+ HER2- BC	Tamoxifen/Fulvestrant/Exemestane + Atezolizumab + Targeted therapies	Phase 1/2	NCT03280563 MORPHEUS	N=111, ORR	Oct 2022
HR+ HER2- BC, premenopausal	Exemestane+ Leuprolide (GnRH analog) + Pembrolizumab	Phase 1/2	NCT02990845	N=25, PFS	Dec 2019
HR+ IBC	Tamoxifen/Aromatase inhibitor/LHRH agonist (physician's choice) + Pembrolizumab	Phase 2	NCT02971748	N=37, DFS	Jan 2020
HR+ BC or TNBC	Anti-estrogen + Pembrolizumab vs Pembrolizumab+ Doxorubicine	Phase 2	NCT02648477	N=56, Safety, ORR	Sep 2018
AR+ TNBC	Pembrolizumab + Enobosarm (Selective AR modulator)	Phase 2	NCT02971761	N=29, Safety, ORR	Oct 2018
mCRPC	Enzalutamide + Atezolizumab vs Enzalutamide	Phase 3	NCT03016312	N=730, OS	Jul 2022
mCRPC	Enzulatamide + Pembrolizumab	Phase 2	NCT02312557	N=58, PSA response	Jan 2019
mCRPC	Enzalutamide + Pembrolizumab vs Pembrolizumab	Phase 2	NCT02787005 KEYNOTE 199	N=370, ORR	Mar 2020
mCRPC	Enzalutamide + PROSTVAC-F/V-TRICOM vs Enzalutamide	Phase 2	NCT01867333	N=57, TTP	Jan 2019
mCRPC	Enzalutamide + Spileucel-T Concurrent vs sequential administration	Phase 2	NCT01981122 STRIDE	N=52, T cell response	NA

mCRPC	Abiraterone acetate (CYP17 inhibitor)+ Prednisone + Ipilimumab	Phase 1/2	NCT01688492	N=57, PFS; safety	Sep 2018
CSPC	Enzalutamide (AR antagonist) + PROSTVAC-F/V-TRICOM vs Enzalutamide	Phase 2	NCT01875250	N=38, Tumor growth	Jan 2019
CSPC, adjuvant or after recurrence	Degarelix (GnRH antagonist)+ Ipilimumab	Phase 2	NCT02020070	N=16, PSA response	Dec 2018
Localized PC, neoadjuvant	Degarelix+ Cyclophosphamide+GVAX vs Degarelix	Phase 1/2	NCT01696877	N=29, CD8+ T cell infiltration, adverse events	May 2019

Table 2 : Clinical trials combining anti-estrogen or androgen deprivation therapy with immunotherapies in breast and prostate cancer, respectively.
ER+: Estrogen receptor positive, BC: breast cancer, HR+: hormone receptor positive, OS: overall survival, TTP: time to progression, CR: complete response, DFS: disease free survival, IBC: inflammatory breast cancer, TNBC: triple negative breast cancer, AR+: androgen receptor positive, mCRPC: metastatic castration resistant prostate cancer, CSPC: castration sensitive prostate cancer, PC: prostate cancer, NA: not available

Hormonal factors

- Aging induced decline
- Pregnancy induced changes
- Pharmacological therapies:
- Menopausal hormone replacement therapy
- Oral contraception

Sex differences in anti-cancer immunity

Genetic factors

-X chromosome located immune genes

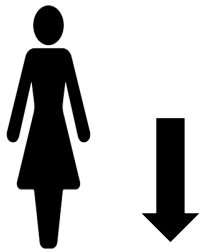
-miRNAs

Environmental factors

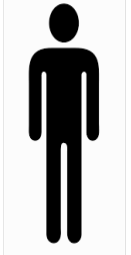
-Chemicals

-UV light

-Ozone



Cancer susceptibility



Anti-estrogen therapies ? Response to immune checkpoint inhibitors ? Anti-androgen therapies

Different immune escape mechanisms ?

Disseminated tumor cells → Late relapse of hormone sensitive cancers ?

Microbiome ?

Figure Legends

Figure 1 Sex hormones, genetic and environmental factors contribute to sex differences in anticancer immunity.

The crosstalk between sex hormone signalling 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 pharmacological 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 anti-estrogen or anti-androgen therapies could improve responses to such therapies.

It remains to be determined whether these observed sex differences in immmunotherapy responses are possibly due to differences in the predominant immune escape mechanisms in tumours 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.