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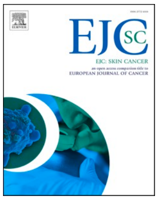

Sex-specific and sex hormonal-related differences in melanoma microenvironment

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ABSTRACT

Growing interest has emerged in the role of sex, as a biological variable that influences the immunopathogenesis of melanoma. Female patients exhibit significantly better disease outcomes compared to males, and antitumor immune responses and benefits of immunotherapy also differ between males and females. Preclinical and clinical studies suggest an association with a more immunogenic tumor microenvironment in women. However, the extent of sex-related differences in immune responses in melanoma remains unclear, highlighting the need for further research. Sex hormones play a role in determining the nature of melanoma tumor microenvironment, as estrogens and androgens have been shown to modulate immune responses, contributing to the differences observed between males and females. The generally more reactive and efficient female immune system may contribute to better antitumor responses. Conversely, males tend to have higher cancer incidence, potentially due to inherent immune-cellular and molecular variations. This review summarizes key findings on the influence of sex on melanoma in general, and more specifically on the melanoma tumor microenvironment and antitumor immune responses, to provide a comprehensive understanding of how sex influences melanoma progression and patients outcomes. Understanding these differences is crucial for developing more effective, personalized treatment strategies for melanoma patients.

1. Introduction

Pathophysiological differences between men and women may influence various diseases, including cancer [1]. In recent years, increased awareness of the importance of sex and gender as key biological and sociocultural variables has been highlighted, in 2016, by the National Institute of Health to urge researchers to consider sex as a biological variable in their experimental projects (SABV) [2]. Biological sex differs from gender, which refers to behaviors and activities shaped by societal or cultural norms in humans [3]. Gender roles, behaviors, and access to healthcare can influence exposure to risk factors, diagnosis, and treatment outcomes. This highlights the importance of considering gender in alongside with biological sex in cancer research and clinical practice. A large variety of cancers, including lung cancer, thyroid, colorectal and esophageal cancer, and primary brain and central nervous system tumors show marked differences in their prevalence according sex [4]. These epidemiological differences in most cases (lung, esophageal cancer, liver and urinary bladder cancer) reverberate in similar difference in mortality rates [4]. Increasing evidence has pointed to both gender- and sex-specific differences regarding the incidence and the prognosis (progression and survival) of melanoma [5]. In this narrative review, we report key findings on the influence of biological sex on antitumor immune responses and on the cellular populations within the melanoma tumor microenvironment (TME).

1.1. *Gender and sex differences: epidemiology and prognosis*

Cutaneous melanoma (CM) is the most frequently diagnosed type of skin cancer. In 2022, the estimated number of prevalent cases (1-year) across both sexes was 136,248 (45.0 %) in Europe, 136,248 (45.0 %) in North America, and 19,514 (6.4 %) in Asia, according to data reported by the World Health Organization [6]. Epidemiological data show that among younger adults, CM is more common in women, whereas among older adults, it is more common in men [7]. These findings are consistent

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with those of Olsen et al., who reported that in eight different populations, women exhibited higher rates of melanoma than men in early life, whereas men had higher rates in later life across all countries studied [8]. As report ed by *American Cancer Society* men are 2.5 times more likely to develop melanoma than women within the same age group [9]. However, women tend to receive melanoma diagnoses more frequently than men before the age of 50 [10]. The reason for the difference is unclear, although it has been attributed to the higher prevalence of tanning activities among females [10]. Nevertheless, in general men appear to be more affected by melanoma. Indeed, a recent analysis in the American population estimated new cases of melanoma of the skin showing sex-related differences, with males accounting for 58.79 % of cases and females for 41.20 % [11]. The male-to-female incidence ratio has increased over time in some countries, from 1982 to 2018, a trend associated with a higher prevalence of head and neck melanoma, and to a lesser extent, trunk melanomas in men compared to women [8,12].

Historically, melanoma was found to predominate in men on the trunk and in women on the limbs, but recent years have witnessed an increase in trunk melanoma among women [12]. Olsen et al., analyzing long-term melanoma data from 1982 to 2018, observed higher rates of trunk, head, and neck melanomas in males across most age groups, with these rates increasing with age [12]. Conversely, females showed a higher prevalence of lower limb melanomas up to middle age, with this prevalence decreasing with advancing age [12]. Considering age, time periods, and birth cohorts the same authors found that environmental changes influenced by behavioral and cultural factors have caused significant shifts in melanoma incidence over time, varying among different genders [12]. However, despite the aforementioned changes in overall incidence rates, age strongly influences sex differences in the anatomical distribution of cutaneous melanoma [12]. In fact, besides sun exposure, sex-specific factors influencing melanocytic proliferation may contribute to these differences in melanoma distribution [8,12]. Indeed, sex differences in anatomical distribution appear to reflect the sex-specific distribution of nevi observed in childhood and adolescence [8,12]. However, it remains uncertain to what extent these differences are due to different patterns of sun exposure versus inherent variations in how melanocytes respond to sunlight between sexes. Melanoma occurring on the trunk in men and on the lower limbs in women may be primarily influenced by genetic factors and less by sun exposure, which is more common in younger age groups [8]. In contrast, melanoma localization on the head, neck, and upper limbs may be associated to cumulative sun exposure, which tends to occur more frequently in older age groups [8].

Therefore, biological sex and gender have become significant risk factors in melanomagenesis, with men being at greater risk than women [5]. These findings suggest a complex interplay between genetic and environmental factors specific to each site, potentially contributing to the observed age-, sex-, and gender-related mortality trends, as prognosis is highly dependent on the location of melanoma [13,14].

Women generally appear to be linked to more favorable outcomes across various cancer subtypes [15]. More specifically, research conducted in Europe and the United States revealed that the advantage was significantly more pronounced in melanoma compared to other tumors [16]. This phenomenon was first reported in the late 1960s, in a cohort of 106 females and 98 males CM patients, by the observation that melanoma was more aggressive in men than in women [17].

A poorer prognosis of primary CM was associated with older age and male gender. Younger patients typically experience a more favorable prognosis compared to older patients, with the difference being particularly pronounced in women [18]. In 2024, the American Cancer Society has estimated mortality rates for melanoma skin cancer highlighting significant sex-specific differences. Males represent 65 % of deaths compared to 34.5 % for females [11]. Some studies suggest that the survival advantage for females may diminish with aging [19] and with increasing metastatic tumor burden [20].

Furthermore, Lasithiotakis et al. reported that female gender was independently associated with extended disease-specific survival (DSS) and survival after recurrence (SAR). In particular, women exhibited significantly lower rates of recurrence compared to men, with female gender being associated with higher rates of local and regional recurrences and a decreased occurrence of distant metastasis [18]. Moreover, research based on melanoma cases from the Munich Cancer Registry indicated that primary melanomas in females displayed a reduced probability of metastasis onset [21]. Indeed, Joosse and colleagues [21], using data from the Munich Cancer Registry, found lower dissemination and consequently lower metastases in lymph nodes and distant sites in women.

Overall, these data suggest a prognostic value of biological sex, indicating variations in the interaction between the tumor and the host among men and women. However, it is important to note that women often demonstrate more health-protective behaviors. This non-biological factor can substantially impact the observed female-positive trend [19]. According to some studies, although differences in lifestyle and behavior may contribute to the delay and higher disease stage in men at diagnosis, the survival advantage in females persists even when accounting for these and other variables such as histological subtypes, Breslow thickness, and body site [20,22]. Furthermore, although thicker and ulcerated tumors are found more frequently in men, Joosse et al. suggested that these factors alone do not fully explain the more unfavorable prognosis observed in men compared to women [23]. In contrast, Afshar et al., studying a population which included 5833 women and 6780 men aged 15–70 years, identified factors such as tumor thickness and tumor site as contributors to the sex-related gap survival. In this cohort men had lower survival rates compared to women (Fig. 1), confirming previous results. Specifically, they found that in 44 % of cases, the difference in survival between two sexes could be attributed to tumor thickness, while in 20 % of cases, it was attributable to tumor site [24].

1.2. Sex hormones

Sex-differences associated with the greater advantage of female sex in survival, are various. They include hormone levels (estrogens and androgens), the immune system, autophagy, matrix metalloproteinase-2, skin physiology, vitamin D, obesity, and reactive oxygen species [1]. These factors collectively influence the progression and survival of melanoma, contributing to sex disparities [16]. Furthermore, sex likely impacts melanoma onset through a combination of genetic factors, including DNA damage response, mutational burden, and genomic instability [26].

Several studies have suggested that X-linked genes play a role in sex-based differences in cancer outcome, including melanoma [16]. Sex chromosomes have been implicated in sex-specific variation regarding general disease susceptibility, immune responses, and disease progression [5]. Interestingly, while approximately 10 % of microRNAs in the human genome are located on X chromosome, only about 2 % reside on Y chromosome [27]. The miR-221&222 and the miR-506–514 cluster, situated on the X chromosome, may potentially contribute to sex differences, in progression of melanoma and transformation of melanocyte [28], although their specific roles have yet to be fully elucidated.

Although melanoma is traditionally considered a non-hormone-dependent tumor, growing evidence suggests a direct correlation between estrogen and melanoma growth and progression [29,30].

Estrogen signaling has been shown to inhibit melanocyte proliferation, enhance differentiation, and suppress melanoma development in experimental models (Fig. 2) [31,32]. An association between estrogen levels, estrogen receptor expression in melanoma, and survival in women has been proposed [33]. However, conflicting results regarding the association between circulating estrogen levels and the initiation and progression of melanoma have been reported [32,34], probably due difficult-to-control oscillations of estrogen levels during the menstrual cycle, onset of menopause, use of oral contraceptives, and hormone

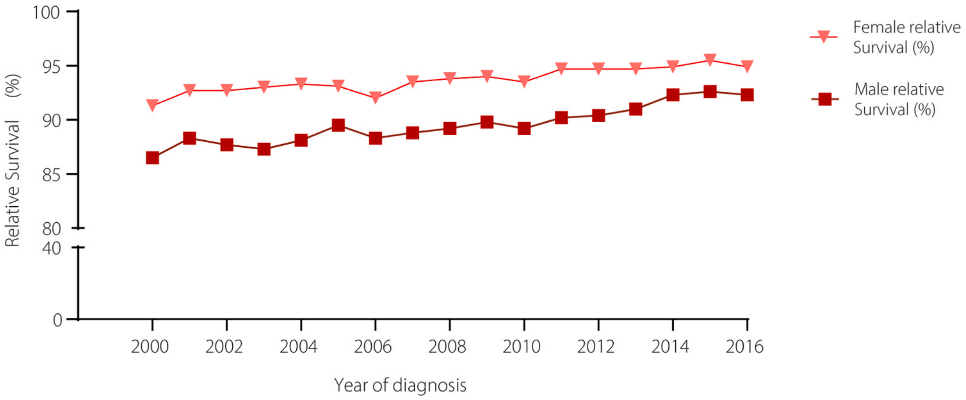

Fig. 1. Melanoma of the skin. Recent trends in SEER Relative Survival Rates, 2000–2021 by Sex, 5-year Relative Survival, all races / ethnicities, all ages, all stages. Modified by GraphPad from SEER*Explorer: An interactive website for SEER cancer statistics [Internet]. Surveillance Research Program, National Cancer Institute; 2024 Apr 17. [updated: 2024 Jun 27; cited 2024 Jun 28]. Available from: https://seer.cancer.gov/statistics-network/explorer/. Data source(s): SEER Incidence Data, November 2023 Submission (1975–2021), SEER 22 registries (excluding Illinois and Massachusetts). Expected Survival Life Tables by Socio-Economic Standards [25].

replacement therapy [35].

Estrogen receptors (ERs) exert contrasting effects on tumor proliferation. Some studies suggest that cutaneous ER are higher in women than in men [36,37], and downregulation of this receptor seems to worsen disease progression [38]. ERα has a tumor-inducing effect and plays a role in estrogen-mediated proliferation whereas ERβ has an antiproliferative effect [39] and pro-apoptotic effects (Fig. 2). ERα is the primary ER in human skin, while ERβ is the dominant receptor in melanocytic lesions. The expression of ERβ decreases as melanoma progresses, supporting its role as a tumor suppressor [40,41]. Notably, ERβ expression levels are lower in men than in women in both melanoma and healthy tissues, as indicated by data obtained from comparisons with adjacent healthy skin [37]. This finding aligns with sex differences observed in melanoma patient survival [21,37], and led to the proposal that malignant transformation is promoted by an upregulation of ERɑ and a downregulation of ERβ [5]. ERβ expression has been shown to be inversely correlated with Breslow thickness, a fundamental predictive marker in melanoma [42]. On the other hand, immunohistochemical analyses have shown that ERβ is present in melanocytic nevi and malignant melanoma cells, while ERα is not [41,43]. Of note, age can influence changes in the immune landscape mediated by hormonal factors. Research on how biological aging differs between sexes has only recently started to appear. Variation in sex hormones linked to sexual maturity represents an interesting variable in this context [5].

In females, investigations on the link between reproductive factors, exogenous hormone use and melanoma incidence have shown that an earlier age at menarche and a later age at menopause are associated with an increased risk of melanoma. This supports the hypothesis that estrogen exposure in childhood may contribute to photocarcinogenic effects [44]. However, the same study found that exogenous estrogen use, as well as parity and age at first live birth, were not associated with an increased risk of melanoma [44]. Similarly, a population-based retrospective study of Yuk and colleagues reported no significant influence of hormone replacement therapy (HRT) on melanoma incidence in menopausal women [45]. Indeed, there is limited evidence available to assess a causal relationship between oral contraception, hormone therapy, and melanoma incidence [46].

The different hormonal signaling pathways present in men and women contribute to the development of specific TME in the two sexes. Consequently, another potential source of sexual dimorphism in CM is the TME.

1.3. *Melanoma TME*

Within the TME a complex ecosystem characterized by reciprocal cell-cell interaction may orchestrate the development of new phenotypes [47]. Sex differences observed in both cancer cells and components of the TME arise from to the interplay of a large variety of factors, which include endogenous sexual hormones [48,49], sex chromosomes [50,51], genetic factors [52,53], epigenetic modifications [50,54], and lifestyles [48,55,56].

On the other hand, TME composition is influenced by the interaction between tumor-related and host-related factors. Specifically, the differential regulation of gene circuits influenced by sex hormones leads to different interactions between tumor and host factors, leading to a sex-dependent evolution of the tumor [57]. TME components may affect cancer cell activity *via* genetically regulated programs, such as apoptosis or autophagy, that the cell deploys under stressful circumstances. Although the role of autophagy in tumor growth remains unclear, the impact of estrogen on autophagy regulation appears crucial for the initiation and advancement of various cancer types [58]. TME components appear as potential targets for personalized approaches in cancer therapy [47,59,60]. By examining how sex can influence TME and the ensuing progression of melanoma tumors, new sex-based biomarkers may support stratification of melanoma patients for a more personalized precision medicine.

1.4. *Cancer associated fibroblast and sexual dimorphism in melanoma*

The stroma supporting CM comprises fibroblasts, endothelial cells, immune cells, soluble molecules, and the extracellular matrix (ECM) [74]. Among these, fibroblasts are the most abundant stromal cells. They interact with other cells of the TME by producing several mediators, including growth factors and ECM components. During CM development, healthy fibroblasts undergo a phenotype transformation to cancer-associated fibroblasts (CAFs) [75]. CAFs play a crucial role in tumorigenesis [47], supporting tumor growth and facilitating the dissemination of metastases [76]. Furthermore, CAFs promote angiogenesis [77], and regulate the immune infiltration [78,79] by remodeling the ECM [47,80].

Although sex hormones, such as estrogens and androgens, have been proposed to control CAF role in tumorigenesis and tumor growth [81, 82], thus differently influencing tumor development in men and women, the role of sex hormone-dependent CAF in tumor progression remains unclear. Regarding the role of estrogens and androgens in cancer-associated fibroblasts (CAFs), conflicting results have been reported in different types of cancer [83]. The variable preclinical or clinical results on the androgen receptors (ARs) role can be attributed to the different species and experimental settings. Most preclinical studies have indicated that the level of AR expression by CAFs correlates with

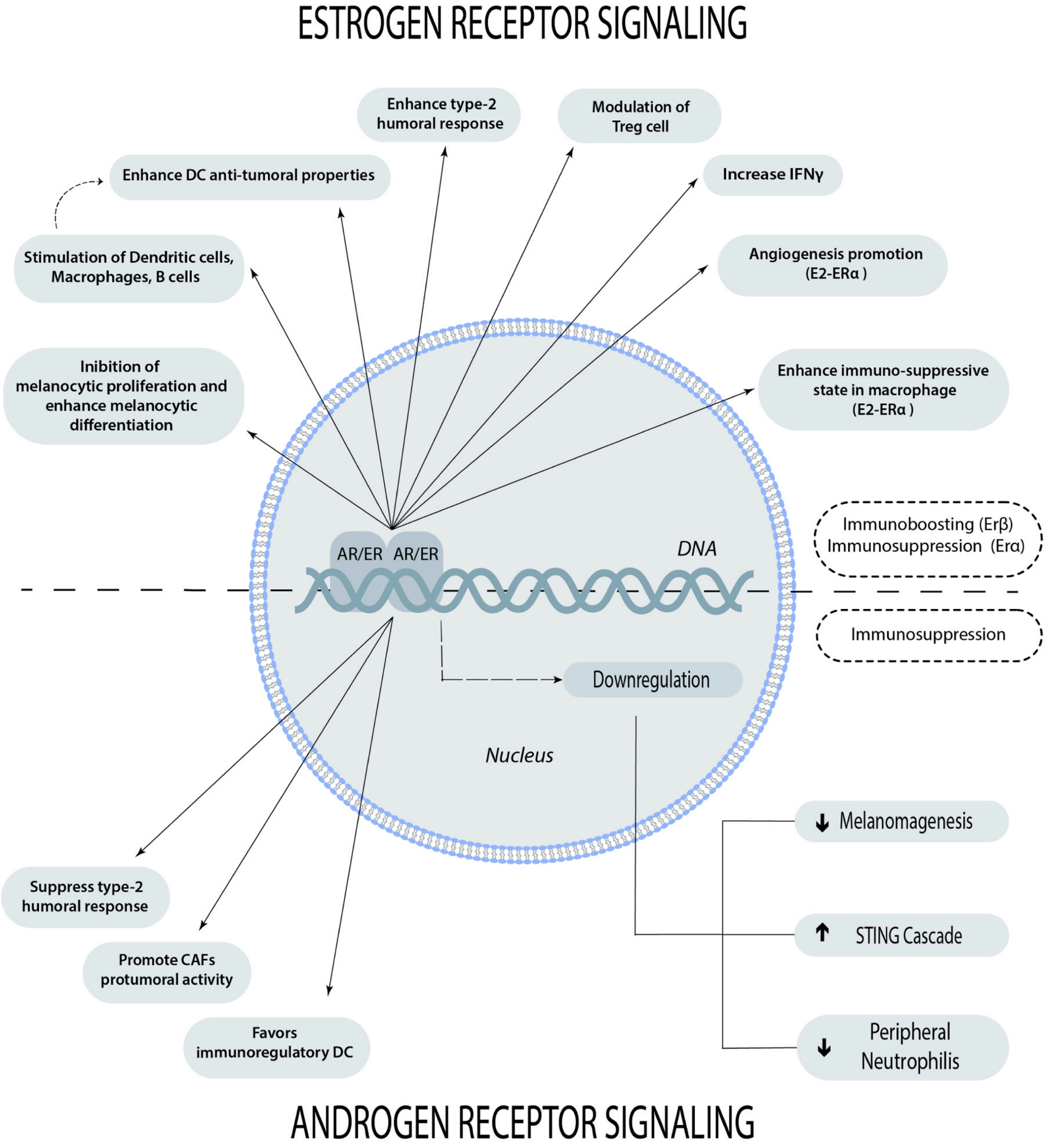

Fig. 2. The influence of sex hormones on immune cell functions and tumor responses. This figure illustrates the complex roles of sex hormones in tumor biology, emphasizing estrogen and androgen signaling. *Estrogen Signaling:* ERα promotes tumor growth and cell proliferation, while ERβ has antiproliferative and pro-apoptotic effects [39]. Estrogen signaling inhibits melanocyte proliferation, enhances differentiation, and suppresses melanoma development in experimental models [31,32]. They act as immune stimulators, affecting macrophages, and B cells, and promote anti-tumoral properties in DCs [61,62]. In females, estrogen and progesterone enhance type-2 humoral responses while repressing type-1 cell-mediated responses (CD8+ T cells) [62]. Estrogens modulate Treg function [63] and enhance IFNγ secretion by upregulating the Th-1 transcription factor, T-bet [64,65]. E2/ERα signaling enhances endothelial cell proliferation and migration [66,67] and drives macrophages toward an immune-suppressive state within the melanoma TME [68]. ***Androgen Signaling:*** testosterone generally has immunosuppressive effects, with inconsistent patterns for type-1 responses and suppression of type-2 responses [62]. Androgens favor the development of immunoregulatory DCs [61]. In preclinical models, castration or androgen inhibition increases tumor burden and alters peripheral neutrophil levels [69]. AR downregulation or pharmacological inhibition reduces melanoma development and increases infiltration of macrophages and cytotoxic T cells [35]. Impaired AR function activates the STING proinflammatory signaling cascade, which is crucial for immune cells infiltration into tumors [70–72]. AR downregulation in fibroblasts promotes early CAF activation [73]. Overall, estrogen signaling tends to enhance immune responses and suppress tumor growth, while androgen signaling has complex and context-dependent effects on immune regulation and tumor progression.

the pro-tumoral activity, favoring tumor progression, metastasis spread, and resistance to therapy [1]. In an *in vitro* model with human cells, Clocchiatti et al., (2018) found a downmodulation of AR expression in human dermal fibroblasts as well as in CAFs (Table 1) derived from the three major skin cancer types: squamous cell carcinomas (SCC), basal cell carcinomas (BCC) and melanomas underlies premalignant skin cancer lesions (actinic keratoses and dysplastic nevi) [73]. Furthermore, downregulation of the AR receptor in human dermal fibroblasts was shown to promote the initial steps of CAFs activation (Fig. 2), thereby enhancing the tumorigenicity of SCC cells and melanoma cells in an orthotopic skin cancer model [73]. Additionally, it was demonstrated that restoring AR levels blocked the tumor promoting effects of CAFs on adjacent cancer cells [73]. The results of this study are not sufficient to demonstrate that androgen signaling causes sex-specific differences but

Table 1
Key findings on sex and sexual hormones related differences in major cell types of the TME in melanoma.

Cell type	Main findings	Ref.
CAFs	Downregulation of AR promotes CAFs activation in an in vitro model	[73]
	Aged fibroblasts increase *AXL* expression, promoting metastasis in aged male mice	[84]
Neutrophils	The absence of testosterone signaling leads to an immature, pro-tumoral phenotype of neutrophils in preclinical model	[69, 116]
CD4+/CD8+ cells	Higher levels of CD4+ helper and CD8+ cytotoxic T lymphocytes, and a lower CD4/CD8 lymphocyte ratio in both blood and tumors in female mice compared to male mice	[5]
	Higher level of CD8+ in males, and CD4+ in females in humans	[3]
Treg cells	Lower frequency of Treg infiltration in female tumors in both preclinical and clinical studies	[3,61]
	Treg function is modulated by both B7-H1 and estrogens in preclinical model. Enhanced antitumor immunity is observed in B7-H1 -/- female mice compared to male mice, which can be attributed to reduced Treg functionality	[63]
DCs	Reduced infiltration and functionality of tolerogenic FOXO3-expressing DCs in females compared to males in vivo	[61]
Macrophages	The Estrogen/ERα axis promotes immune-suppressive macrophage polarization in the melanoma TME in preclinical model	[68]
	AR downregulation increases macrophage infiltration into tumors	[35]

they highlight its importance in both sexes. In this context, it is crucial to further investigate sex-specific differences in the formation of CAFs and their role in melanoma, given their significant role in tumorigenesis. On the other hand, a recent study revealed that dermal fibroblasts undergo sex-specific changes with aging, which in turn differentially affect melanoma cells. Specifically, aged male fibroblasts promote metastasis in older male mice by increasing *AXL* expression [84]. Additionally, aging in male fibroblasts, driven by a decline in *EZH2* which led to an increase in *BMP2* secretion, fosters a highly invasive melanoma cell phenotype, characteristic of the aging male TME [84].

1.5. *Neoangiogenesis and sexual dimorphism in melanoma*

The formation of new blood vessels (neoangiogenesis) supplying cancer tissue is a complex process which requires several factors and cell types, besides cancer cells, such as fibroblasts, immune cells, and endothelial cells [85]. Although in CM tumorigenesis, especially when TME become hypoxic, this process plays a major role, to date, a limited number of studies have specifically investigated the impact of sex on tumor angiogenesis in melanoma [86].

In an *in vitro* study E2 (17-β-estradiol) or Erα signaling in endothelial cells from female donors produce proliferation and migration properties superior to male endothelial cells (Fig. 2) [66,67]. Endothelial cells isolate from the vein of the umbilical cord (HUVECs) of male newborns produce more H2O2, thereby increasing the cellular oxidative stress [66, 67]. The known reduced activity of that antioxidant enzymes in males, might be responsible for the higher levels of oxidative stress compared to females [34]. Oxidative stress is associated with cell autophagy [66,67], and endothelial cell autophagy plays a crucial role in modulating vascular biology and functions, including survival in response to metabolic stressors, maintaining redox homeostasis, and regulating vessel permeability.

In tumoral context, autophagy has been shown to promote angiogenesis and tumor growth [87]. The use of ERα antagonists, such as tamoxifen and 1-methyl-4-phenylpyridinium (MPP), reduces the synthesis of VEGF-A and FGF-2, tumor volume, and angiogenesis in female but not in male mice [88]. VEGF-A and FGF-2 are growth factors implicated in cell proliferation by binding to their receptor expressed on the surface of endothelial cells [89,90]. E2 promotes the growth of ER-negative melanoma tumors in vivo, by increasing VEGF-A synthesis, promoting and normalizing tumor angiogenesis, thereby favoring blood supply to prevent tumor hypoxia and necrosis [88]. This process relies on the expression of ERα by stromal endothelial cells.

1.6. *Immunity and sexual dimorphism in melanoma*

It is well-established that TME significantly influences the development and progression of CM [91]. Both sex and gender could influence the immune response [3]. Sex differences in the immune system, resulting from sex hormones activities and different genetic backgrounds between the two sexes, affect both innate and adaptive immunity [3]. Female immune system predominantly features tumor-associated antigen-specific T cells (TAA-T), B cells, and antigen-presenting dendritic cells (DCs), while male immune system is characterized by natural killer (NK) cells and regulatory T cells [3] (Fig. 3). Recent research highlights sex-specific differences in immune system regulation [57], with the female immune system that exhibits greater reactivity and efficiency compared to its male counterpart [92, 93]. Women generally exhibit enhanced immunity against various infectious agents, and an incidence of autoimmune diseases higher than men [57]. Accordingly, cancer incidence may differ between females and males not only because of genetic, epigenetic and environmental factors, but also because of the different cellular and molecular immune responses between the two sexes [94]. Sex chromosomes, whether X or Y, have several genes encoding proteins crucial for immune function. The X chromosome is rich in genes that play crucial roles in immune regulation, significantly affecting sex differences in immune-related diseases. Notable genes include Toll-like receptor 7 (TLR7), various cytokine receptors such as interleukin-2 receptor subunit gamma (IL2RG) and interleukin 13 receptor subunit alpha 2 (IL13RA2), and transcription factors like Forkhead Box P3 (FOXP3) [95,96]. Furthermore, the X chromosome harbors approximately 10 % of the human miRNA repertoire, including miRNA-18 and miRNA-19, which may influence immune responses [27]. Due to incomplete X inactivation, females may express higher levels of these miRNAs, which could contribute to the observed sex differences in disease susceptibility [3].

Hormonal receptors such as ER, AR, and progesterone receptor (PR) are present in various immune cells [97,98], influencing both innate and adaptive immune responses [99,100]. Specifically, ERs and AR are expressed in both T and B lymphocyte populations [101,102] where they regulate estrogenic and androgenic signaling pathways. Testosterone tends to exert immunosuppressive effects, whereas estrogens are generally regarded as immunoboosting (Fig. 2) [62]. However, it should be underlined that the role of AR signaling in melanoma remains poorly understood [35]. The binding of androgens with their receptor results in immunosuppressive response in various cell types including macrophages, neutrophils, B cells and T cells [103]. Conversely, estrogens have been identified as immune stimulators, particularly on dendritic cells, macrophages, and B cells [62] (Fig. 2). Therefore, androgens seem to counteract the action on immune and protective role of estrogens [100,104].

Downregulation or pharmacological inhibition of AR decreased the development of melanoma (Fig. 2), resulting in increased infiltration of macrophages and cytotoxic T cells into the intratumoral compartment (Table 1) [35]. The STING (stimulator of interferon genes) proinflammatory signaling cascade is activated when AR function is impaired (Fig. 2), which is essential for enabling immune cell infiltration within the tumors [70–72]. This mechanism enhances the immune system ability to recognize and eliminate cancer cells, potentially benefiting a significant proportion of melanoma patients [108]. Enhancing the response to immune checkpoint inhibitors (ICI) could be achieved by inhibiting the AR, especially in tumors categorized as

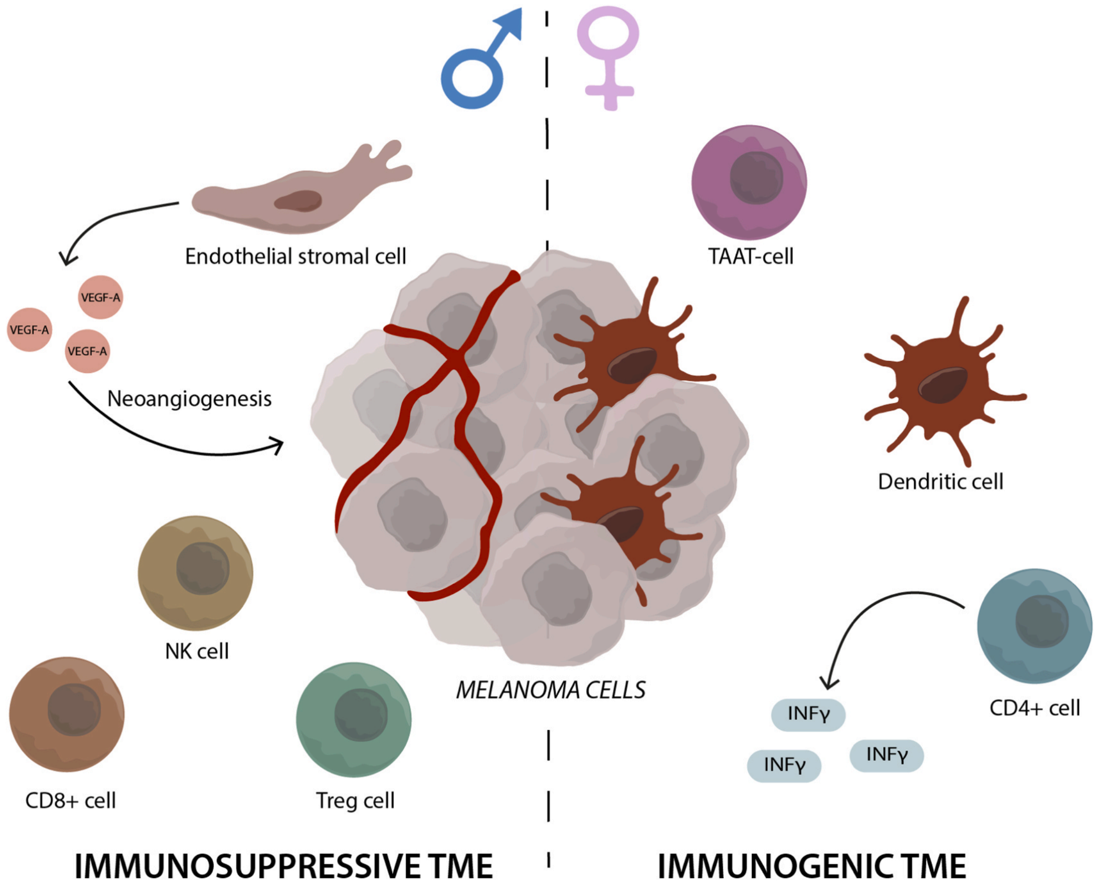

Fig. 3. Sex-specific differences in immune cell populations involved in melanoma. Fig. 3 illustrates the predominant immune cell populations observed at higher frequencies in males (i.e., CD8+, Treg, NK cells) [3], and endothelial cells [105] on the left. The latter contributes to neoangiogenesis through the release of VEGF-A. In female patients, the immune phenotype is enriched in TAA-T cells, CD4+, and DCs, with elevated levels of circulating IFN γ [3,106,107], on the right. These different populations influence the antitumor response, with females displaying a more efficient antitumoral immune response and males showing an impairment in antitumor immune response and an angiogenic phenotype. NK: natural killer; TAA: tumor-associated antigen; DCs: dendritic cells.

"immune-excluded" or "immune desert" [109,110]. This approach may exploit sex hormone differences to improve treatment outcomes [111].

Regarding estrogen signaling, a robust immunoreactivity of ERβ (with weak ERα) has been reported in melanocytic lesions located in the epidermis, papillary dermis, and periphery of dermal aggregates [43]. Conversely, a marked reduction in immunoreactivity was found in nodules located deeper in the dermis [43]. IFN-transcription and secretion can be boosted by estrogens, resulting in the promotion and expansion of T-cell populations and responses (Fig. 2) [3,97]. The Estrogen/ERα axis has been identified as a key driver to pushing macrophages toward an immune-suppressive state within the melanoma TME (Fig. 2; Table 1) [68]. The ER status, which controls the infiltration of lymphocytes, is considered a predictive marker of cancer survival and clinical outcome. Estrogens are hypothesized as major players in the sex disparity in melanoma [112]. Taken together, these data show that sex steroids are potent regulators of immune response contributing to disease onset and progression of diseases in melanoma [1]. Despite the effects of sex hormones on immune response, it is still not clear whether these effects are subject to sex-specific biases, as androgens, like estrogens, are expressed in both sexes. For instance, one study has reported a negative correlation between AR activity and immune infiltration in both men and women, with sex-specific differences depending on tumor type [113]. In melanoma, AR levels appear similar in both sexes [114]. In this context, a study has identified that the origin of sexual dimorphism may lie in the intersection between the non-canonical transcriptional repertoire of AR and oncogenic protein fucosylation, which facilitates melanoma invasiveness potentially during both early-stage establishment and late-stage metastatic progression in androgen-responsive melanomas [114]. Moreover, in a recent paper it was found that global tumor fucosylation is significantly lower in male compared to female melanoma patients, suggesting a sex-associated divergence in fucosylation-regulated melanoma biology [115]. Liu and colleagues outline a mechanism that seems to explain how androgen signaling/AR shapes melanoma malignancy, enhancing invasive and metastatic capacity by inducing tumorigenic fucosylation, thus contributing to the male bias in melanoma.

Consequently, it is crucial to further study the impact of sex hormones and if and how they influence a sex-correlated immune response in melanoma. This may involve more than just considering receptor or hormonal expression levels, implicating complex mechanisms and intersection with other factors.

1.7. Neutrophils

Although the main function of neutrophils is to identify and by phagocytosis destroy foreign and harmful microorganisms, their role in cancer has been investigated also in the context of sex differences. AR are identically expressed in female and male neutrophils [103]. The protective effect of androgens against tumor and metastasis development has been linked to the direct or indirect actions in neutrophils. In metastatic mouse models, the crucial role of testosterone signaling in neutrophils has been demonstrated [69]. Specifically, when AR signaling is absent in the bone marrow, neutrophils maintain an immature phenotype which is associated with a pro-tumoral profile

(Table 1) [116]. Mice deficient in AR exhibit neutropenia, confirming the importance of the hormonal environment in the differentiation, maturation, and proliferation of neutrophils [117,118]. In preclinical models, castration or androgen inhibition has been shown to increase tumor burden in melanoma. Castration of male mice resulted in a decrease in the percentage of peripheral neutrophils (Fig. 2; Table 1), which was restored upon administration of testosterone. Neutrophil depletion decreased activation of NK cells and increased tumor burden [69]. In this context, it is crucial to further investigate whether the androgen/AR pathway leads to sex-specific differences in melanoma, particularly in relation to neutrophil maturation. On the other hand, a study in the murine melanoma cell line B16F10 revealed a sex-dependent dimorphism of liver metastasis (LM), linked to an enhanced accumulation and function of pro-metastatic neutrophils in the liver, mediated by androgen/AR signaling [119]. This mechanism could contribute to the male-biased sexual dimorphism in LM observed in human populations. The signaling pathway specifically increased neutrophil production by promoting the proliferation and development of neutrophil precursors in the bone marrow [119].

1.8. *Lymphocytes*

1.8.1. *CD4+ and CD8+ cells and different ratio between female and male*

The crucial role played by T lymphocytes in melanoma is supported by large and robust evidence. Specifically, T helper cells are pivotal in defending against malignant tumor, through recruiting and activating antigen-specific effector cells [120]. CD4+ cells are essential in the initial stages of T-cell mediated responses, while CD8+ cells become critical during the effector stage. During these responses, cytokines such as IFNγ and interleukin-2 (IL-2), which play crucial roles in anti-tumor immunity, are released [121]. CD4 and CD8 are also key because they orchestrate the adaptive immune response, a primary line of defense against melanoma [5]. The ability of CD8+ T cells to recognize antigens that deviate from normal cells in abundance or molecular structure allows them to eliminate malignant cells with remarkable efficacy [122].

It is now well recognized the existence of a sex-dependent disparity in the adaptive immune response, contributing to differences in tumor progression between males and females. The more efficient female immune system has a particularly evident function in the context of melanoma [3]. Additionally, significant sex differences in CD8+ T cell-mediated anti-tumor immunity have been reported [122]. In this context, sexual hormones influence T-cell mediated responses. Exogenous estrogen has been shown to enhance the expansion of regulatory T cell populations in vivo in a mouse model [123]. In addition, estrogens enhance IFNγ secretion by increasing its transcription or upregulating the Th-1 transcription factor, T-bet, which promotes T cell responses and cell-mediated immunity [64,65] (Fig. 2). Furthermore, female mice produce higher levels of IFNγ by helper T cells in response to parasitic infections, compared to male mice [124]. Even in humans, females produce higher levels of IFNγ upon stimulation of naïve CD4+ T cells compared to males [106]. However, other findings suggest that estrogens can inhibit Th1-mediated proinflammatory cytokine secretion. Despite this, female melanoma patients were found to have higher frequencies of tumor-specific-antigen Th1 cell compared to males [107]. In females, estrogen and progesterone enhanced type-2 humoral response and repressed cell mediated type-1 response (CD8+ T cells), whereas testosterone suppressed type-2 response and showed an inconsistent pattern for type-1 response (Fig. 2) [62]. Thus, preclinical and clinical evidence suggests a qualitative and quantitative difference in the composition of the TME between males and females.

Using a syngeneic B16-F10/BL6 melanoma mouse model, Dakup and colleagues (2020) explored the impact of biological sex on melanoma progression. They found that female mice exhibited higher levels of CD4+ helper and CD8+ cytotoxic T lymphocytes along with a lower CD4/CD8 lymphocyte ratio in both blood and tumors compared to male mice (Table 1). Female mice also demonstrated a higher infiltration rate of CD8+ T cells, which correlated inversely with tumor volumes, leading to decreased tumor progression [125]. A lower CD4/CD8 T cell ratio was associated with a positive prognosis of tumor progression and favorable survival outcomes. These findings suggest that the adaptive T cell response to melanoma is heightened in female mice compared to male mice, corresponding to sex differences in tumor growth. However, a recent brief report indicated that in the BrafCA; Tyr-CreERT2; Ptenf/f mouse model, melanoma developed more rapidly in female mice than in male mice, both in terms of initiation and progression [126]. Additionally, Thompson et al. (2017) found that in a model mice of melanoma tumors grew slower in males compared to females [61]. These discrepancies between female and male mice suggest that sex-specific signaling and/or mechanisms could play a significant role in the development of melanoma [126].

In humans, females were reported to have a higher CD4+ T cell population and a higher CD4/CD8 ratio than males, whereas a higher CD8+ T cell population was found in males (Table 1) [3]. A higher frequency of CD4+ tumor associated antigen-specific T cell (TAA) cell was found in female patients compared to their male counterpart (Fig. 3) [107]. Additionally, the composition of immune cells was found to vary significantly across different tumor types in relation to sex. Considering T-cell population, the relative abundance of activated CD4+T-cells was found in women with lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma, esophageal carcinoma (ESCA), and stomach adenocarcinoma (STAD). Conversely, the relative abundance was higher in men with kidney renal clear cell carcinoma (KIRC), adrenocortical carcinoma (ACC), pheochromocytoma and paraganglioma (PCPG) and skin cutaneous melanoma [127]. Sex-related differences in tumor-infiltrating lymphocytes (TIL) were not observed in a retrospective study involving over 14,000 patients [128].

1.8.2. *TReg lymphocytes*

Treg cells, by promoting immune self-tolerance and inhibiting tumor immune surveillance, have a fundamental role in tumor immunopathogenesis [129–131], and their function in cancer may exhibit sex differences. In both healthy and chronically inflammatory conditions, females consistently exhibit lower and less potent Treg cells population [132,133]. Regulatory T cells are a therapeutic target of ICIs, given their high expression of immune-inhibitory receptors such as CTLA-4 and PD-1. Studies in mice investigating sex disparity in Treg cells reveal conflicting findings regarding their organ-specific frequencies across various diseases, whereas human studies indicate higher counts of Treg cells in healthy adult males compared to females [132]. In mice, a lower frequency of Treg infiltration in female tumors has been reported (Table 1) [61]. Additionally, in a murine model (murine melanoma B16), differences in antitumor immunity and response to immunotherapies have been observed depending on both sex and B7-H1 expression [63]. Treg function, mediated by B7-H1, is modulated by estrogens (Fig. 2; Table 1). B7-H1 (PD-L1) is a co-signaling molecule expressed widely by immune cells [134].

Evidence suggests that B7-H1 inhibits antitumor immunity, thereby contributing to immune evasion [130,135] and plays a significant role in regulating Treg function. A study highlighted that female mice lacking B7-H1 (B7-H1 -/-) exhibited enhanced antitumor immunity due to reduced functionality of Tregs compared to male mice (Table 1) [63]. Moreover, after B7-H1 blockade, the response to immunotherapies in wild-type (WT) female mice was more effective than in WT male mice. The sexually dimorphic response to B7-H1 blockade underscores that the enhanced efficacy of anti-B7-H1 therapy in reducing Treg function in WT females may underly the role of B7-H1 signals in sex-based differences of antitumor immunity. Notably, the lower B7-H1 expression levels in WT female mice as compared to WT males, were not associated with alteration in Treg suppression in the presence of estrogen *in vitro* [63]. Conversely, Treg cell suppression in B7-H1 -/- female mice, exhibited sensitivity to estrogen *in vitro*, leading to reduced Treg suppression [63]. This observation implies that these effects occur before the final differentiation of Tregs. Li and colleagues [63] illustrated that these phenomena do not rely on PD-L or CD80 binding, proposing a novel mechanism in the B7-H1 signaling pathway, concluding that depleting Tregs via B7-H1 blockade could provide greater advantages in females, particularly when combined with an E2 agonist as part of antitumor immunotherapy [63].

1.9. Dendritic cells

Dendritic cells (DCs) are a subset of antigen-presenting cells crucial for immune responses. Hormone signaling significantly impacts the development of these cells [136]. In melanoma TME DCs are responsible for regulating T cell activity and contributing to immune infiltration which is a measure of immune-therapy response [137]. According to Thompson et al., (2017) the effectiveness of antitumor immunity may not solely depend on the overall quantity of dendritic cells within TME, but rather on whether these cells exhibit an immune-activating or immune-tolerizing phenotype. Estrogens can also indirectly influence the responses of dendritic cells. In female mice engrafted with melanoma and breast tumors, there is reduced infiltration of tolerogenic transcription factor FOXO3-expressing DCs which are also less functional compared to their counterparts in male mice (Table 1). The FOXO3 plays a critical role in DCs tolerance and immunosuppression [61]. Interestingly, it has been observed that FOXO3 regulates the expression of the AR specifically in males by binding to its promoter region. This interaction triggers a positive feedback loop that promotes the polarization of DCs into a tolerogenic phenotype [61]. Overall, this implies that androgen favors immunoregulatory DCs while estrogens favor DCs anti-tumoral properties, which can be advantageous for female cancer survival rates (Fig. 2). Recent studies have shown significant differences in melanoma tumor growth between male and female mice, correlating with variations in the infiltration of specific immune cell populations such as DCs and T cells [61].

2. Conclusions

The TME and its components may be influenced by sex, making them important general biomarkers for cancer therapy and precision medicine. Despite the increasing number of studies considering biological sex in melanoma research and TME studies, there are some limitations in the above mentioned studies.

The study by Dakup and colleagues (2020) presents a potential confounding factor, indeed the endocrine system and particularly sex steroids can affect immune responses by binding to hormone receptors expressed on immune cells [125]. Similarly, in the study conducted by Zhai, a potential bias could be the use of tamoxifen for the induction of melanoma, indeed tamoxifen is a selective estrogen receptor modulator and may exert differential effects in male and female mice [126].

Although there are data on the role of sex hormones and their receptors within the TME, these findings do not consistently translate into clear sex-specific effects. Therefore, further studies are needed to explore whether sex hormone pathways differ between two sexes, despite their expression in both sexes, and to determine whether the differences observed between the two sexes are independent of hormonal signaling. Is AR activity increased in melanoma, given its immunosuppressive action? If so, are there differences between sexes?

Understanding these differences could reveal how they contribute to the development of distinct TMEs and influence tumor progression. Given the limited evidence regarding the relationship between oral contraception, hormone therapy, and melanoma incidence [46], it is of the utmost importance to elucidate the role of these factors in melanoma development and to inform preventive strategies. Furthermore, there is a lack of data on how these factors might influence the composition of the TME.

These findings suggest that the immune system could be a major factor contributing to sex differences observed in melanoma, with females exhibiting greater antitumor immune activity compared to males. However, the mechanisms underlying sex disparities in melanoma remain mostly unclear and, further investigation is necessary to characterize circulating and tumor-infiltrating T cells [125] and to elucidate the mechanisms underlying sex differences in the initiation, progression, and survival or mortality rates among melanoma patients [5].

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CRediT authorship contribution statement

Filippo Ugolini: Writing – original draft, Methodology, Investigation. **Selene Attorre:** Writing – original draft, Methodology, Investigation. **Daniela Massi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Maria Grazia Giovannini:** Writing – review & editing, Supervision. **Daniele Lana:** Writing – original draft, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

All authors critically reviewed the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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