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The gender perspective in cancer research and therapy: novel insights and on-going hypotheses

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Abstract
Cancer represents a leading cause of death whose incidence is steadily increasing worldwide due to the population aging. The Global Health Observatory of the World Health Organization reported that approximately 13% of all deaths are caused by cancer. In the 2012 the estimated total number of cancer deaths was 1.75 million, 56% in men and 44% in women. Gender is recognized to play a role in cancer incidence, progression and response to therapy. Besides anatomical and hormonal disparities, genetic differences should be considered when assessing the effects of gender on cancer. Accumulating evidence also support the existence of sex-driven differences in immune responses.
Until today clinical trials and research in animal models have been gender unbalanced. In consideration of the differences between sexes observed in cancer, sex should represent an important stratification factor to be included in all randomized clinical trials for a better understanding of biological differences between men and women, which may yield improved targeted therapies.

Key words
- gender
- cancer
- immune therapy
- sex
- epidemiology
- toxicity

INTRODUCTION

Cancer represents a leading cause of death whose incidence is steadily increasing worldwide due to the population aging and to lifestyle behaviors, as smoking, overweight and physical inactivity. Gender disparity in the incidence, aggressiveness and disease prognosis have been observed for a variety of cancers. Despite this, clinical trials and research in animal models have been gender unbalanced. Gender-specific oncology needs to reconstruct a balance in order to understand the molecular basis underlying the gender differences in the outcome and response to therapy of cancer. The comprehension of the biological mechanisms responsible for sex-biased differences may yield improved cancer management and the development of personalized therapeutic strategies.

The words *sex* and *gender* are quite distinct. In fact, *sex* is referred to the biologic characters that distinguish males and females as expressed by the analysis of the person's gonadal, morphologic (internal and external), chromosomal and hormonal features. By contrast, *gender* refers to behaviors, roles, expectations and activities in society. In other words, gender refers to socio-cultural or learned significance of *sex*. The current distinction between *sex* and *gender* difference has been criticized as misleading and counterproductive, especially in the field of medicine. In this review we decided to use the terms gender and sex interchangeably.

GENDER DISPARITIES OF CANCER: EPIDEMIOLOGY AND MOLECULAR BASIS

Growing evidences are showing gender-associated functions playing a role in cancer incidence, progression and response to therapy. At present cancer represents the leading cause of death, approximately 13% of all deaths, as reported by The Global Health Observatory of the World Health Organization (WHO). Cancer incidence and mortality for 2012 were estimated by sex and age groups for each of the 39 European countries, defined by United Nations (UN) plus Cyprus.

According to the European Network of Cancer Registries (ENCR) member registries, the WHO mortality database and UN population data, the amounts estimated in 2012 in Europe by type of cancer were over 3.4 million of new cases (excluding non-melanoma skin cancers), 53% (1.8 million) occurring in men and 47% (1.6 million) in women. The most common cancer sites were breast cancer (464 000 cases, 13.5% of all cancer

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cases), followed by colorectal cancer (447 000, 13.0%), prostate cancer (417 000, 12.1%) and lung cancer (410 000, 11.9%), representing half (50.5%) of the estimated overall burden. In the same year the estimated total number of cancer deaths was 1.75 million, 56% in men and 44% in women.

Based on these numbers and considering the differences observed in tumor location, incidence, aggressiveness and response to therapies, it appears of outmost importance to introduce a gender-derived approach in cancer research. Moreover the comprehension of biological and socio-cultural specificities associates either with sex or gender would provide better results in prevention and therapeutic strategies. Nonetheless, really few preclinical studies use animals of both sexes to investigate the molecular mechanisms underlying cancer development and results derived from clinical trials are not evaluated taking in mind sex and gender.

Besides anatomical and hormonal disparities, genetic differences should be considered when assessing the effects of gender on diseases. Different studies showed the female advantage in several different cancer types. Based on EUROCARE-4 cancer cases, in Europe a significant advantage of women was reported for 16 out of 26 types of cancer [1]. Representative examples of gender disparities have been associated with colorectal cancer, urothelial and kidney cancer [2] as well as melanoma [3].

Colorectal cancer, the third most common cancer in the world, is characterized by sex- and gender-specific differences, as women appear more prone than men to right-sided colon cancer, a more aggressive form of this neoplasia. Depending on tumor site, colorectal cancer is associated with different molecular and pathological characteristics: microsatellite instability (MSI) and BRAF mutation often observed in right-sided colon cancer and chromosomal instability more often in left-sided (60%-70% of cases) [4]. Hormonal factors might explain a large percentage of right-sided colorectal cancer in females as estrogen appears a protective factor against MSI as suggested by the increased risk of MSI-high colon cancer in older women. Accordingly the Women's Health Initiative Clinical Trial reported that postmenopausal women undergoing hormone replacement therapy showed a 40% reduction in colorectal cancer risk [5]. In addition, besides sexual differences in anatomy and physiology of the colon (longer transverse colon in women), socio-cultural differences, as dietary factors, should be considered.

A gender discrepancy exists in the incidence of bladder cancer, renal cell carcinoma (RCC) and urothelial carcinoma (UTUC), although few data are available for the latter. Evidences suggest that men present these cancers more than women. Conversely, female gender seems to represent a negative prognostic factor of bladder cancer survival, but it seems to be protective in patients with UTUC or RCC [2]. Smoking habits, occupational risk factors, tumour biology and sex steroid hormones and their receptors could all have a role in causing the observed gender disparities in patients with urological cancers. Interestingly, a meta-analysis of gene expression in clear cell RCC showed that, among the total number of genes analyzed, 89% were differentially expressed between genders [6]. More accurate diagnosis and stratification on higher numbers of patients should be evaluated to actually discriminate the actual sex associated disparities.

Although female sex is associated with a survival advantage in several cancer types, studies in both Europe and United States showed that this advantage was considerably higher in melanoma than in virtually any other type of cancer.

Since the late 1960s, Clark observed that cutaneous melanoma was more aggressive in men [7]. More recently, according to EUROCARE4 data, melanoma was reported to display the more significant female advantage in survival of cancer patients [1]. Based on 1.6 million population, women resulted to have an estimated overall 2% lower relative risk of dying from melanoma in comparison to men and melanoma survival was 50% higher in women compared with men. Seemingly in 2014, in the United States women only accounted for 42% of the new cases and only 33% of the deaths. Further insights were provided on the prognostic effect of sex in a population group of approximately 4000 skin melanomas in central Italy. Data confirmed that women had a 34% lower risk compared with men of dying from skin melanoma [8]. Nonetheless the protective factor is still unknown. Although the source of gender disparity in melanoma remains unclear, two main hypotheses for female advantage can be offered: 1) differences in behavior and 2) unknown biologic sex differences [9].

The most evident behavioral differences are represented by a general earlier detection of lesions due to female more observed health care. Another variation possibly associated with gender is the site of primary melanoma, more often on the extremities for women and on the trunk for men. Melanomas localized on the legs could be more easily visible respect to the back, but more exposed to UV [10].

Looking for the underlying molecular mechanisms, tumor itself does not really appear to diverge across gender as confirmed by several studies showing no differences in mutation rates of some important genes in melanoma, most notably in the BRAF gene. Nonetheless several host factors, which differ across gender have been linked to melanoma progression and survival and might therefore offer an explanation for the female melanoma advantage. These include hormone levels (estrogens and androgens, estrogen receptor β), the immune system, autophagy, matrix metalloproteinase-2, skin physiology, vitamin D, obesity and reactive oxygen species. In fact all these molecules and biological processes have been hypothesized to display sex disparities *(Figure 1)*. As an example, it is known that males express lower amounts of anti-oxidant enzymes, resulting in more oxidative stress than females [11]. On the contrary, melanoma incidence and progression do not seem actually influenced by estrogens as the female survival advantage seems to persist in postmenopausal age [12].

Melanomas in women have a lower propensity to metastasize, resulting in a better survival when compared with men, thus suggesting differences in tumor-host interaction across gender. In fact women display a

significantly lower risk of both lymph node and distant metastases when compared with men, even after adjustment for relevant prognostic factors. Indeed melanoma is a highly immunogenic tumor, likely inducing immunosuppression, and gender is known to influence the immune system [13].

Finally several studies suggest a role for X linked genes in sex-based cancer outcome differences, including in melanoma. A key factor in X-chromosome inactivation is DNA methylation thus indicating that epigenetics contribute to sex-specific metabolic phenotypes [14]. Although in women X chromosome undergoes inactivation of one copy in each cell, due to escape or variation of inactivation X chromosome, heterogeneity does exist and female tissues are mosaics. Conversely, men are identically monosomic for the maternal X chromosome.

Finally, particularly intriguing appear sex differences associated with microRNAs, whose deregulation has been significantly associated with cancer progression. MicroRNAs are small non-coding RNAs involved in the post-transcriptional regulation of up to 70% of protein-coding genes. In addition, differential microRNA expression has been reported in males and females and their possible contribution associated to sex disease outcome. Differences in the expression of microRNAs can depend on a differential regulation by sex chromosome genes, as the X chromosome shows a high density of microRNAs, 2-fold higher than autosomes. According to miRBase microRNA archive (www.miRBase.org 2013), the X chromosome encodes 113 microRNAs and the Y chromosome only 2. Hence sex differences in microRNA expression seem a result of both hormonal and genetic differences [15]. Possible examples are miR-29a and -29b induced by estrogens in women and protecting against liver fibrosis by reducing collagen deposition associated with cancer dissemination [16]. Also, in view of their localization on the X chromosome, miR-221&222 as well as the miR-506-514 cluster could be related with sex differences. Oncogenic roles have been demonstrated for both these microRNA groups in melanoma progression and melanocyte transformation [17].

SEX DIFFERENCES IN IMMUNO-ONCOLOGY AND ANTICANCER IMMUNOTHERAPY

Accumulating evidence support the existence of sex-driven differences in immune responses as potential factors contributing to diverse disease outcome as well as response to therapies in males and females [18]. The sex-bias in the immune response is due to diverse determinants: the direct effects of X- or Y-chromosome linked genes, the indirect effects of sex hormones and the environmental risk factors that act in a sex-specific manner [19]. X-linked genes as well as unique genes on the Y chromosome play a crucial role in the divergence between male and female immune responses. On this basis, the X chromosome may be considered partially responsible for the immunological advantage of females that are characterized by increased immune reactivity. In fact, numerous genes, directly and indirectly involved in immunity, are encoded by the X chromosome, conferring a sex-based advantage not only due to the prevention of the deleterious effects of heterozygous-linked gene-mutations but also to the benefit of a greater diversity appropriate to face new immune challenges, such as infections or vaccines [20]. Accordingly, mutations in some X-linked immune genes, such as the IL-2 receptor gamma chain or FOXP3, lead to severe dysregulated immune phenotypes in males, while females result relatively unaffected [19]. Moreover, being miRNAs critical regulators of the development of immune cells and of the maintenance of immune system homeostasis, their unbalanced massive presence on the X chromosome implies a potential dysregulation associated to female-specific immune diseases [21]. The second main cause of sex-bias differences in immunity is the influence of sex hormones on immune cells. In fact, through specific receptors, sex hormones control the development and function of multiple immune cell populations, shaping innate and adoptive immune responses [22]. Estrogens regulate inflammatory pathways of macrophages in a dosage-dependent manner and suppress neutrophils; they also have a remarkable impact on the differentiation and activation of dendritic cells (DCs), including important DC subsets target for immunotherapies, such as plasmacytoid DCs (pDCs) [23]. In this regard, it has been recently reported that sex differences of IRF5 in pDCs drive higher IFN-α production in women than in men [24]. Estrogens increase NK cytotoxicity and the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α whereas androgen exert opposite effects on NK cells and stimulate the production of IL-10 [25]. Importantly, women have on average higher frequency of circulating $CD4^+$ T-cells then men [26]. Estrogens influence the maturation of T lymphocytes and regulate T-helper (Th)1 and Th2 responses in a dosage-dependent manner, since low doses promote Th1-mediated immunity, whereas higher doses skew the immune response towards Th2. In addition, they regulate Th17 lineage polarization and drive the expansion and the functional capacity of Tregs. Indeed, these cells are dramatically sensitive to changes in the levels of sex hormones during the ovarian cycle, since their frequency increase in the follicular phase upon estrogen level enhancement and decrease during the luteal phase when estrogens go down and progesterone is high. Thus, sex hormones exert an additional control on effector T cells throughout Treg, determining the outcome of adaptive immunity. B cell development and humoral immune response are also shaped by estrogens that drive the enhancement of B cell subsets and control the levels of immunoglobulins (Ig), in particular IgG and IgM. In fact, one of the most evident sex-specific immune differences is the ability of females to produce more elevated circulating levels of antibodies than males, attesting their capability to produce much stronger humoral immune responses [27]. Regarding the control of sex hormones on immune cells, recently it has been described the capability of these factors to alter the epigenetic regulation of gene expression by affecting epigenetic modifications, in particular DNA methylation and chromatin remodeling, thus adding an important level of influence on the differentiation and the activation of immune cell popu-

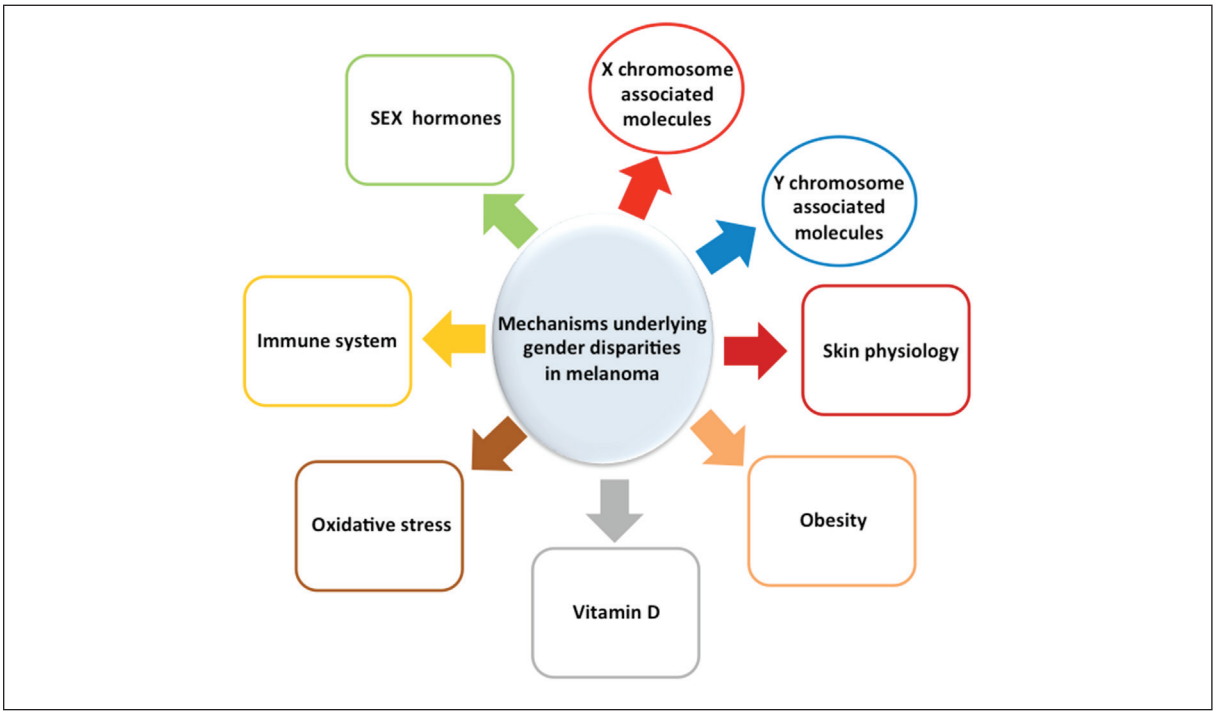

Figure 1
Biological processes possibly contributing to sex disparities in melanoma. The schematic picture shows the main mechanisms associated with women better outcomes.

lations. Thus, a sex-specific epigenetic mechanism for immune modulation is also envisaged [23]. The third factor concurring to determine sex-specific immunity is a complex network of environmental components (see *Figure 2* for a schematic description of sex differences in immune phenotype and in anticancer response to immunotherapy). Although the exposure to antigens, such as pathogens, chemicals and xenobiotics, and food intake and variety are important in orchestrating immune responses, the microbiome-related factors seem to be the most important source vigorously influencing the expression of sex-specific immune phenotypes. Recently, it has become evident a tight dialogue between the commensal gut microbiome and host hormones. In this bidirectional interaction, host hormones control microbiome composition and, in turn, the microbiome influences sex hormone levels of the host shaping hormone-dependent immune responses [28]. In addition, sex hormones control gut local immunity shaping a crucial crosstalk between immune and epithelial cells. In fact, estrogens activate in situ a wide range of pro-inflammatory signals, including differentiation of IL-12-producing DC, polarization of Th1/Th17, B cell activation and activation of Toll-like receptors pathways. These events concur to generate pro-inflammatory environment which determine the dysfunction of gut permeability allowing the translocation of gut bacteria into the lamina propria, that in turn engages the amplification of pro-inflammatory responses. Altogether, sex-associated factors determine specific immune phenotypes displaying deep differences in male and female with the consequence that women generally exhibit more robust immunity against infections, higher allograft rejection and increased rate of autoimmunity than men [29]. Surprisingly, in this scenario the sex-driven immune response to anticancer treatments targeting the immune system remains the less investigated issue in the field.

The antitumor immunity is fundamental in shaping the outcome of several anticancer therapies, including conventional chemotherapy and radiation therapy, because behind their direct main action driven by tumoricidal properties toward fast-dividing cancer cells, these treatments exert profound effects on immune cells within the tumor microenvironment (TME) mainly by recruiting tumor-infiltrating leukocytes to this site upon rebound effects, that in turn regulate the overall immune response to therapy. However, the antitumor response driven by effector $CD8^+$ T cells is often dampen by the immunosuppressive microenvironment in place mediated by a multitude of immune cells and counterregulatory mechanisms [30]. By targeting these components, cancer immunotherapy has shown to elicit remarkable long-lasting responses in patients with a variety of advanced cancer as compared with conventional chemotherapy. In this landscape, the sex disparity in eliciting antitumor response with a specific immune phenotype may have a dramatic impact on the efficacy of cancer immunotherapies. This assumption is fundamental in the evaluation and implementation of the recently developed immune checkpoint inhibitors (ICI), that represent a pillar of cancer therapy, since they have revolutionized treatment and outcome of severe and often fatal cancers, with long-term tumor control and extended patient survival [31]. Nowadays, three ICI (ipilimumab, nivolumab, pembolizumab) and one (nivolumab) are treatments of choice for melanoma

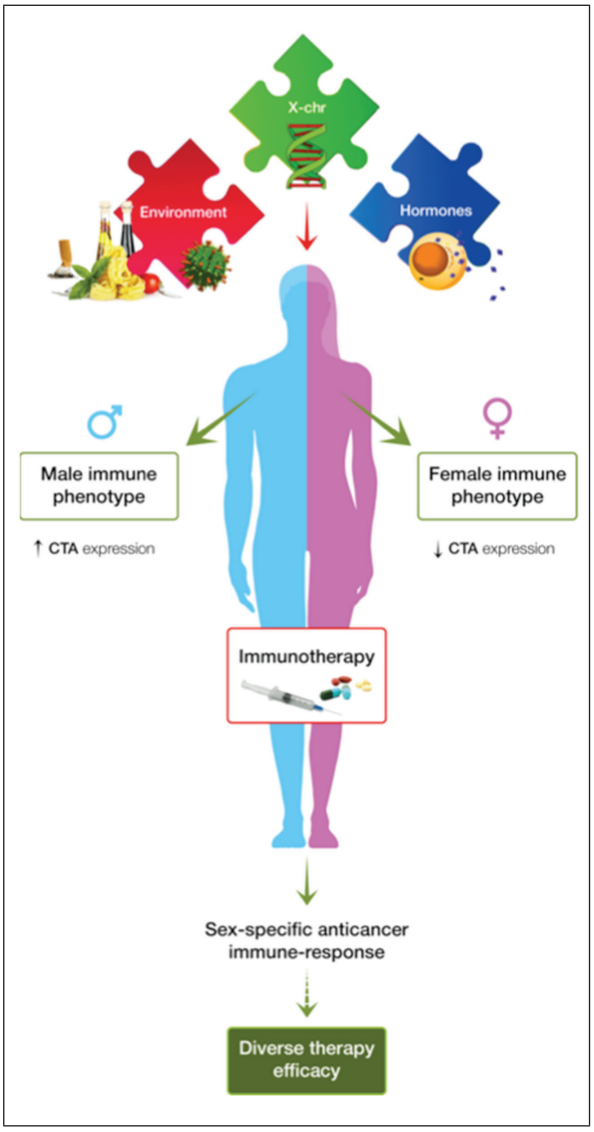

Figure 2
Influence of sex immune phenotype on anticancer response to immunotherapy. Specific determinants such as X-chromosome linked genes, sex hormones and environmental risk factors act in a sex-driven manner. The integration of the out coming signals determines the sex-biased immune-phenotypes that in combination with other elements, for instance the differential expression of CTA, shape diverse anticancer responses in males and females, affecting immunotherapy efficacy.

and non-small-cell lung carcinoma, respectively. Nevertheless, since ICI affect T-cell function, treatment-associated side effects have challenged oncologists with a novel spectrum of toxicity, causing reduced dosage, delayed drug administration and therapy discontinuation. In this regard, a fundamental aspect still totally unexplored is the sex disparity in immune and toxic responses and their relationship. Importantly, among toxic effects, one of the most severe is colitis, due to the change in microbiome, causing dysfunction of intestinal barrier [32]. These evidences necessarily recall the importance to take into consideration the sex-driven differences in microbiome composition.

In spite of the growing body of indications suggesting the leading role of sex-bias in the response to cancer immunotherapy, the absence of knowledge in this field is largely due to two major causes, specifically animal researches performed with only one sex and under-enrollment of women in clinical trials [33]. Nevertheless, some studies suggest important mechanisms by which sex is likely to affect the response to immunological-based anticancer treatments, such as anti-PD1-based immunotherapy. PD-1 receptor is expressed by active T cells and when it is bound to PDL-1, aberrantly expressed by cancer cells, it suppresses the immune response of T cells. By blocking PD-1 receptors with anti-PD-1 mAbs, T cells are free to respond to cancer antigens and attack tumor cells [34]. Importantly, it has been shown that estrogens enhance Treg suppressive activity, throughout PD-1-dependent and PD-1- independent pathways leading to significant differences in tumor immunity and immunotherapy responses in males and females [35]. Accordingly, B16 melanoma-bearing female mice have been found to benefit more than the male counterpart from anti-PD-L1 therapy, partially due to greater PD-L1 blockade-mediated reduction of Treg function in females [36].

In the framework of immunotherapy another critical issue is the definition of tumor specific antigens toward which the immune response needs to be redirected. The ideal condition is to have antigens expressed exclusively in cancer cells rather than normal tissues. In this view, cancer-testis antigens (CTA) represent promising candidates for immunotherapy since they are never expressed in any normal tissue besides testis but aberrantly present in many types of human cancer. Hence, the identification of novel CTA is considered a prerequisite for the development of new cancer vaccine strategies [37]. Importantly, some CTA expressed in lung cancer, such as MAGE3 and NY-SAR-35, were found to be significantly associated with male sex, whereas, the expression of Ropporin-1, a potential CTA target for multiple myeloma immunotherapy, seems remarkably higher in females [38]. Hence, the differential expression of CTA in males and females needs to be taken carefully into account in order to develop effective immunotherapeutic strategies.

Overall, given the magnitude of sex components driving immunity, the disparity between males and females in eliciting both innate and adoptive immune responses is crucial for defining the response to anticancer treatments whose efficacy relies on the competence of the immune system. Hence, taking into account the sex issue in the design of anticancer immunotherapeutic strategies, and more in general of all therapies that target the immune system even only partially, is mandatory and represents a challenge as well as an opportunity for optimizing cancer treatments, including the management of therapy-related toxicity.

GENDER DISPARITIES IN EFFICACY AND TOXICITY OF ANTICANCER THERAPY

Although gender disparity in the incidence of cancer, the aggressiveness and disease prognosis have been observed for a variety of cancers, relatively little is known

and assessed about the gender differences in anticancer therapy and their impact in the clinical management of the disease. The low representation of women in clinical trials certainly represents a crucial factor that has limited, to date, the collection of data. In the last 30 years, too many epidemiological and clinical studies reported results in only one sex. This means that results obtained only in men, in terms of efficacy and toxicity, were transferred to the entire population, including women [39]. This aspect is very important for the implications either in drug-related toxicity or efficacy.

Knowledge of the potential benefits and risks associated with the use of anticancer therapies is fundamental for making treatment-related recommendations and decisions. Emerging data from the literature about gender differences are partial, fragmentary and sometimes contradictory. Due to the retrospective nature of these studies, there are numerous confounding factors (age, stage of disease, co-morbidity etc.) that may have impacted results.

In general, it has been observed that some chemotherapy protocols lead to a better response rate in women without increasing toxicity (*e.g.* cisplatin and irinotecan), while others only increase toxicity, but do not improve response rates in women (*e.g.* 5-fluorouracil). Side effects appear to be highly dependent on different tissue properties, as women have a higher incidence of oral mucositis, but lower rates of gut toxicity than men. Nausea and vomiting is a greater problem in females during therapy due to the lower activity of anti-emetic drugs [40].

While the molecular mechanisms underlying these differences are not yet or only partially known, the increased toxicity often correlates with different pharmacokinetics.

Gender differences in drug pharmacokinetics and pharmacodynamics have been recognized to play a key role in drug efficacy and safety profile [41]. Although gender-related pharmacodynamics data are limited, evidence suggests that women i) are more prone to the development of side effects than men and ii) present different pharmacological response to drug treatment that could translate into a different clinical outcome.

Physiological differences between male and female exist in tissue specific metabolism. The liver is one example. Sexual differences have been described for hepatic transport, as well as for enzymatic activities, drug detoxification and lipid metabolism [42]. For example: i) many detoxifying enzymes belonging to the cytochrome P450 (CYP) superfamily are expressed in the liver in a sex-dependent pattern; and ii) CYP3A4 and CYP2B6, responsible for the metabolism of more than 50% of therapeutic drugs, exhibit higher activity in women than in men [43, 44]. According to this, women generally predominate among patients with drug-induced liver injury [45] and also they appear to be more susceptible to antineoplastic drug adverse reactions [46].

Sex differences in drug metabolism and elimination are also strictly related to steroid hormone levels. In fact, to further complicate this matter, drug metabolism in women is affected by sex-specific factors such as menopause, pregnancy and menstruation. Thus, careful attention should be paid to the side effects and toxicity arising from sex differences in drug metabolism in different clinical situations. Although there are specific ethical considerations regarding the inclusion of women in drug trials and their inclusion could increase the costs, in terms of both money and work, the relationship between the side-effects and toxicity that may be influenced by hormones during drug metabolism and drug treatment needs to be deeply investigated. However, given the complexity of gender pharmacology as well as the limited availability of adequate animal models and human studies, specific gender disparities are quite difficult to be evidenced.

The following are some examples of gender disparities in toxicity and effectiveness of certain drugs used in the treatment of the most common cancers.

Despite the introduction of innovative drugs and biological agents in cancer therapy, anthracyclines remain among the most potent anticancer drug employed in numerous chemotherapy protocols of both hematologic and solid tumors. However, their use is hindered by the risk of severe cardiotoxicity. According to this, a very recent study reports that clinical use of anthracyclines may lead to a progressive cardiomyopathy that may evolve to congestive heart failure [47]. As far as the gender difference was concerned, most of studies suggested that females develop less severe doxorubicin-induced cardiomyopathy and nephropathy than males [48]. A recent experimental paper, performed by Gonzales *et al.* in tumor-bearing spontaneously hypertensive rats, shows that males were more sensitive to doxorubicin-induced cardiotoxicity. In particular, the authors showed that females exhibited more down-regulation of apoptotic and oxidative stress genes than males after doxorubicin administration. In addition, they also found that reproductive hormone levels were inversely correlated with cardiac health [47]. This is in accord with the fact that the myocardium is functionally responsive to circulating reproductive hormones, as cardiac tissues express both androgen and estrogen receptors [49]. Especially, estrogens have been suggested to function as a cardio-protectant through the prevention of cardiomyocyte apoptosis and alleviation of left ventricular hypertrophy thus providing protection against the development of cardiac fibrosis in women [50]. A further important issue emerging from this paper is the observation of a gender disparity in doxorubicin-induced anticancer activity. In particular, in male animals a significant greater tumor reduction was found in comparison with female animals.

In contrast with that reported by Gonzales and coworkers, Moulin *et al.* did not observed either oxidative stress or cell death as determinant of sexual dimorphism in doxorubicin-cardiotoxicity. In this paper, performed in adult rats, doxorubicin-induced cardiotoxicity, observed only in males, was accompanied by an early mitochondrial dysfunction and altered energy signaling pathways together with cardiolipin homeostasis. These data strongly emphasized the role of mitochondrial dysfunction in doxorubicin cardiotoxicity and further possible role in the observed sex-differences [48].

Amrubicin, an anthracycline derivative, is a completely synthetic agent, which is distinguishable from other anthracyclines discovered through natural products. It has been designed to have an antitumor activity that is from 5 to 200 times greater than that of its parent compound. The use of Amrubicin in phase II trials monotherapy for lung cancer had shown a dose-limiting toxicity due to neutropenia, and severe hematological toxicities [51]. A retrospective analysis to identify pretreatment factors associated with severe hematological toxicity point to, among others, the female gender as significantly correlated with severe toxicity of clinical relevance [52]. Bevacizumab, a monoclonal antibody targeting the vascular endothelial growth factor (VEGF), was approved by the U.S. Food and Drug Administration (FDA) for use in combination with paclitaxel and carboplatin for patients with advanced stage of non-squamous lung cancer [53]. In fact, with the recognition of the importance of angiogenesis in cancer growth and metastasis [54], various therapies have been developed to block this pathway, including antibodies against VEGF. Brahmer and co-worker reported that, in general, subjects treated with bevacizumab experienced antiangiogenesis therapy toxicities such as hypertension and hemoptysis. Comparing males and females, they found that females treated with bevacizumab had a higher rate of grade ≥ 3 hypertension compared to males, while hemoptysis, other bleeding events and proteinuria occurred similarly between males and females. Importantly, whereas both progression free survival and response rate were improved with the addition of bevacizumab either in males or females, bevacizumab was an active drug only in women in which significantly increased the overall survival [55]. This is in accord with the observation that antibodies often have a longer half-life in women, which is associated with an improved response to therapy [40]. By contrast, other trials regarding bevacizumab in advanced colon cancer demonstrated a benefit both in males and females [56].

A better response to melanoma chemotherapy in women treated with dacarbazine in comparison with men was also reported [57] and a specific meta-analysis reports a better response in women than in men when adding tamoxifen to dacarbazine treatment [58]. By contrast, there were no differences in the female survival advantage between patients receiving interferon as well as BRAF V600E inhibitor vemurafenib [59].

Even in children and adolescents gender differences in anticancer drug efficacy and toxicity have been reported [60]. In a study on 352 children treated for anaplastic large cell lymphoma, Wrobel *et al.* observed significantly higher rates of toxicity in females, including grade 4 haematologic toxicity and grade 3-4 stomatitis after treatment with the alkylating agents cyclophosphamide and ifosfamide [61]. It is possible that gender differences in the metabolism of both ifosfamide and cyclophosphamide might contribute to a potential interaction between alkylating treatment, gender, disease outcome and acute toxicity. In fact, both ifosfamide and cyclophosphamide are inactive pro-drugs undergoing hydroxylation as a primary activating step mainly under control of CYP3A4 and CYP2B6, known to differently work in male and female.

Female gender seems also to represent a risk factor to cognitive sequelae in children after treatment of central nervous system cancer. The rate of intelligence quotient decline during anticancer therapy is associated with several risk factors, including younger age at time of treatment, longer time since treatment, clinical variables such as hydrocephalus, use of radiotherapy and radiotherapy dose, and the volume of the brain that received treatment. Anyway, some studies reported that, at equal risk factors, females showed a significantly higher vulnerability to intelligence quotient decline than men [63].

CONCLUSIONS

During the last decades, clinical trials and research in animal models have been gender unbalanced. On the basis of above reported data, it appears evident that sex influences pathophysiology, clinical signs, outcome and therapy of cancer. Thus, sex is an important stratification factor that should be included in all cancer clinical trials for a better understanding of biological differences between males and females, pivotal for improving targeted therapies. However, this new dimension of oncology needs additional investment in research, reform of medical teaching and, most of all, the political determination of changing the health approaches.

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Conflict of interest statement

There are no potential conflicts of interest.

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