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REVIEW

Sex and gender perspectives in colorectal cancer

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Historically women were frequently excluded from clinical trials and drug usage to protect unborn babies from potential harm. As a consequence, the impact of sex and gender on both tumour biology and clinical outcomes has been largely underestimated. Although interrelated and often used interchangeably, sex and gender are not equivalent concepts. Sex is a biological attribute that defines species according to their chromosomal makeup and reproductive organ, while gender refers to a chosen sexual identity. Sex dimorphisms are rarely taken into account, in either preclinical or clinical research, with inadequate analysis of differences in outcomes according to sex or gender still widespread, reflecting a gap in our knowledge for a large proportion of the target population. Underestimation of sex-based differences in study design and analyses has invariably led to 'one-drug' treatment regimens for both males and females. For patients with colorectal cancer (CRC), sex also has an impact on the disease incidence, clinicopathological features, therapeutic outcomes, and tolerability to anticancer treatments. Although the global incidence of CRC is higher in male subjects, the proportion of patients presenting right-sided tumours and *BRAF* mutations is higher among females. Concerning sex-related differences in treatment efficacy and toxicity, drug dosage does not take into account sex-specific differences in pharmacokinetics. Toxicity associated with fluoropyrimidines, targeted therapies, and immunotherapies has been reported to be more extensive for females with CRC than for males, although evidence about differences in efficacy is more controversial. This article aims to provide an overview of the research achieved so far into sex and gender differences in cancer and summarize the growing body of literature illustrating the sex and gender perspective in CRC and their impact in relation to tumour biology and treatment efficacy and toxicity. We propose endorsing research on how biological sex and gender influence CRC as an added value for precision oncology.

Key words: colorectal cancer, gender, sex, tumour biology, treatment, toxicity

INTRODUCTION

With the exception of thyroid cancer, incidence of non-reproductive tumours is higher in males than in females, while mortality rates in males doubling those in females.[1] Sex does not only influence cancer incidence, but also clinicopathological features of disease, differences in treatments, therapeutic outcomes, and tolerability. These sex-associated differences are known as sexual dimorphisms. However, it was not until recently that sex disparities in oncology have been acknowledged.

Although interrelated and often used interchangeably, sex and gender are not equivalent concepts. Sex is a biological attribute that defines species, including humans, as male, female, and/or intersex according to their chromosomal makeup and reproductive organs.[2] Gender, on the other hand, is a chosen sexual identity and represents a social construct that refers to the norms, identities, and relations that structure our societies and organizations, and shape behaviours, products, technologies, environments, and knowledge.[3] Gender is a dynamic concept that varies from society to society and can change throughout an individual's lifetime. Although appropriate reporting of sex and gender in oncology practices is vital, the incorporation of gender variables in clinical research and patients' medical histories remains limited,[4] restricting us from adequately addressing the impact of gender-influenced behaviours on health outcomes, which are different from those influenced by biological sex.

Colorectal cancer (CRC) is the third most common cancer worldwide and the fourth leading cause of cancer death in the world.[1] As in other tumours, there are differences in

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incidence according to sex. Worldwide estimation of incident cases in 2020 was 547 619 and 288 852 cases of colon and rectum cancer in females, respectively, and 600 896 and 443 358 cases of colon and rectum cancer in males, respectively.

This review provides context for understanding sex and gender differences in cancer and summarizes the growing body of literature illustrating the sex and gender perspective in CRC and their impact in relation to tumour biology and treatment efficacy and toxicity.

HISTORICAL OVERVIEW OF SEX- AND GENDER-BASED CANCER RESEARCH

Women were historically largely excluded as subjects of investigations in non-reproductive clinical research, resulting in the extrapolation of data from male-based investigations to women.[5,6] However, underlying and fundamental differences in biology are likely to affect disease development and pharmacokinetics, and impact treatment efficacy and toxicity, which have been widely described.

Following the scandals resulting from the use of thalidomide in women during pregnancy, warnings about fetal risks led to the labelling of pregnant women by the National Commission for the Protection of Human Subjects and Biomedical and Behavioural Research as vulnerable research subjects. In 1977, the United States Food and Drug Administration (FDA) issued a guidance document entitled "General Considerations for the Clinical Evaluation of Drugs" advising that women of childbearing potential should be excluded from early-phase clinical research, with the exception of trials testing drugs for life-threatening illnesses.[7] Women could be included in later phase II and III trials for drugs with a favourable risk—benefit ratio, as long as studies about teratogenicity and fertility had been accomplished. However, the term 'woman capable of becoming pregnant' covered a broad range of women, since it could include premenopausal single abstinent women, women using contraceptives, and women with sterile partners, whereas it did not account for the reproductive desires of women and their partners. Thus, advocacy groups criticized the 1977 FDA guideline by arguing that it deprived women of opportunities, and did not focus on women's independence to make decisions. They also voiced that women were capable of endorsing drug development about sex differences through clinical research participation; furthermore, this policy had an unintended consequence of causing underrepresentation of women in clinical research.

In 1986, the National Institutes of Health (NIH) reinforced the movement by establishing a policy that urged the inclusion of women in clinical trials and finally, in 1993, the FDA reversed the 1977 guidance and lifted the ban that prevented women of childbearing potential from being enrolled in early-phase trials and promulgated "Guidelines for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs". The document pointed out that clinical trial subjects should be representative of the patient population to which the drug would likely be prescribed after approval, and highlighted the importance of exploring differences in terms of safety, efficacy, pharmacokinetics, and pharmacodynamics among subpopulations.[8]

In 1994, the FDA furthered its commitment with the creation of an Office of Women's Health to address the health of women through policy, science, and outreach and to promote the inclusion of women in clinical studies as well as subanalyses of sex, gender, and subpopulations.[9] In 1998, the FDA amended its regulations pertaining to new drug applications (NDAs) and announced the publication of a new document "Presentation of Safety and Effectiveness Data for Certain Subgroups of the Population in Investigational New Drug Application Reports and New Drug Applications" that specifically stated that safety and efficacy data for important populations, including sex, age, and racial subgroups, were mandatory for NDAs.[10] In 2000, the FDA issued the "Investigational New Drug Applications: Amendment to Clinical Hold Regulations for Products Intended for Life-Threatening Disease and Conditions" that permitted placing on hold any trial for a life-threatening condition that excluded patients only because of their reproductive potential.[11]

Although today women are systematically included in clinical trials, inadequate analysis of differences in outcomes according to sex or gender remains widespread and reflects an unmet need deserving of attention and deeper knowledge.

SEX DISPARITIES IN PRECLINICAL RESEARCH

The powerhouse of clinical cancer research springs from *in vitro* workhorse models using cell lines and validation in *in vivo* animal models, including patient-derived models. However, historical ignorance of the role that sex plays across this process has widely jeopardized bench-to-bedside cancer research, obscuring fundamental sex differences that may guide clinical studies.

Male cell lines stocked in repositories of human non-reproductive cancer cell lines outnumber female cell lines, leading to single-sex analyses. Furthermore, few *in vitro* cell-based experiments report the sex of the cell line, posing a potential risk to analysis and interpretation and, even when investigators do acknowledge the sex origin of a cell line, the original sexual identity may transform over the course of routine cell culture passaging.[12,13] Added to this, the sex of cultured cells and that of cell culture media are rarely matched. In fact, the effect of culture media components on the hormonal environment of cultured cells is seldom considered.[14] Hormone concentrations in calf fetus sera may differ depending on whether the serum is sourced from a single sex or a mixture, whereas hormone levels are not routinely measured and sex identity is usually not reported. Finally, the estrogenic contribution of plastic labware commonly used for cell culture or that of the common pH dye indicator phenol red is also rarely considered.[15-17] Phenol red, present in most commercial media, turns yellow in response to acidification of the medium during cell

Table 1. Summary of the complex interplay that the exposome, sex chromosomes, sex hormones, and immunity can have in intrinsic control of cancer-initiating cell populations and cancer development

	Sex and gender disparities in cancer development
Exposome	Exposure to known risk factors for cancer (dietary habits, exercise, smoking, drinking, socioeconomic status, social support, and industrial pollution) can differ according to gender, while strength and direction of the correlation might be dependent on sex and/or gender (i.e. stronger associations between obesity and increased risk of overall CRC have been found in men, compared with women).
Sex chromosomes	X chromosome inactivation process in females is normally random, cells within females have a mosaic expression of either the maternal or paternal chromosome: • EXITS (tumour suppressor genes that escape from X-inactivation) might be responsible for the lower propensity of cancer in females, in comparison with males. • Reactivation of the Xi chromosome due to loss of XIST expression triggers unfavourable genome-wide changes (DNA replication, chromosome segregation, cell cycle checkpoints, and haematopoietic genetic disorders).
Sex hormones	Sex hormones exert pleiotropic functions on multiple tissues. Cancer cells secrete vascular endothelial growth factor A (VEGFA) and platelet-activating factor (PAF) in response to estrogens, enhancing proliferation and migration. ERα is up-regulated in T cells while ERβ is highly expressed in B cells.
Immunity	Approximately 50 X-linked genes, including *FOXP3* and *TLR8*, are involved in adaptive and innate immunity. Females are able to mount stronger immune responses than males and immunity decline against cancer occurs at later age in females.

CRC, colorectal cancer; ERα, estrogen receptor-α; XIST, X-inactive specific transcript.

growth; however, binding and activation of estrogen receptors in multiple cell lines in a dose-dependent manner has been reported.

In the case of *in vivo* experiments using animal models, bias concerning the sex parameter also exists. Species-specific sex differences, and the fact that these vary between species [e.g. that fewer genes escape X chromosome inactivation (XCI) in female mice than in humans, and X-linked gene regulation] should be taken into consideration.[18,19] However, research invariably fails to investigate sex disparity by the differential use of models of male and female animals[20] and furthermore, when using xenografts, investigators seldom account for matching the sex of transplanted cells and their animal recipients, even though this is not consistent with reliable modelling. Plus, as males present aggressive behaviour that requires cage separation, cost concerns often result in favouring a single-sex study in co-caged female mice of premenopausal age, even though human cancers are predominantly diagnosed at a late age.[21,22]

In light of this and striving for the inclusion of both sexes in preclinical research, in 2014 the NIH unveiled a new policy requiring federally funded scientists to include both males and females in cell and animal studies.[23]

SEX AND GENDER DISPARITIES IN CANCER DEVELOPMENT

Exposome

The exposome, defined as the repertoire of exposures and associated interactions of a given individual during their lifetime, may differ among individuals according to their gender. The exposome is known to have an impact on cancer development, with obesity, smoking habits, and inflammation having a direct and strong correlation with carcinogenesis in multiple tumour types[24-26] (Table 1).

Concerning CRC, external exposures such as dietary habits, exercise, smoking, drinking, socioeconomic status, social support, and industrial pollution are known risk factors for colorectal carcinogenesis.[27-35] Exposure can differ according to gender, while strength and direction of the correlation might be dependent on sex and/or gender. As such, stronger associations between obesity and increased risk of overall CRC have been found in men, compared with women. Patterns also differ: weight gain later in life seems to be an important risk factor for CRC in men, while in women, early life obesity is a known risk factor.[36-38] However, to date, sex-specific differences have not been sufficiently investigated for CRC. Findings about dietary patterns, which are related to obesity, and their contribution to CRC have been reported mainly from female-specific prospective cohort studies such as the Nurses' Health.[39] However, unfortunately, large cohort studies that included both women and men mostly have not reported sex-specific estimates. To date, investigations have focused on tumour location and analysed sex differences in terms of risk by colorectal subsite according to high carbohydrate intake, concluding that it might increase right-sided colon cancer in women, while increasing rectal cancer in men.[40] A high inflammatory profile (proinflammatory diet plus sedentarism plus obesity) has been associated with higher risk for colon cancer in men and no significant association in women in the European Prospective Investigation into Cancer and nutrition study (EPIC), while in another study, soy consumption (a known phytoestrogen) was not associated with risk of CRC in males, but risk reduction in females was reported.[41,42]

However, even after adjusting for external exposures, incidence of non-reproductive cancers is lower in females than in males, suggesting sexual dimorphisms.[43,44] In the following sections, we focus on the complex role that sex chromosomes and sex hormones appear to have in this context.

Sex chromosomes and cancer

Male and female development diverges under the influence of both X and Y allosomes (sexual chromosomes) and autosomes (non-sexual chromosomes) and the contribution of sex steroid hormones. However, these sexual dimorphisms might also contribute to sex disparities in cancer development.

Since female cells harbour two entire X chromosomes, a critical XCI to avoid simultaneous expression of two entire X chromosomes must occur at early cell division in implanted embryos. This phenomenon is mediated by the long non-coding RNA X-inactive specific transcript (XIST), which silences one X pair at a random.[45] As a consequence, XX cells present a silenced and inactive X chromosome (Xi) and an expressed and active one (Xa). As XX cells express either the maternal or the paternal X arbitrarily, females and males with Klinefelter syndrome present distinct X gene repertoires, resulting in mosaicism and phenotypic diversity. This contrasts with the exclusive expression of the only X chromosome in XY cells from males.

However, some genes escape and are expressed from both the Xa and the Xi, conferring protective benefits from cancer and other diseases, but also potential risks. X-linked oncogene activation or loss of a tumour suppressor would not be expressed in all cells due to mosaicism, while in males it would lead to obligatory expression of the same alteration in the maternal X-linked gene. Tumour suppressor genes that escape from X-inactivation, termed EXITS, are *ATRX*, *CNKSR2*, *DDX3X*, *KDM5C*, *KDM6A*, and *MAGEC3*, and might be responsible for the lower propensity of cancer in females, in comparison with males.[46,47] On the other hand, the loss of XIST expression may promote tumour development, as the deletion of XIST expression causes the reactivation of the Xi chromosome, triggering unfavourable genome-wide changes, including involvement in DNA replication, chromosome segregation, cell cycle checkpoints, and haematopoietic genetic disorders.[48-50] In males, loss of Y chromosome expression might also constitute a sex-specific biomarker, as it is related to six Y-linked genes known to serve as tumour suppression genes (*KDM6C*, KDM5D, DDX3Y, *EIF1AY*, *RPS4Y1*, and *ZFY*). Reduced transcription levels of these genes have been found in 12 non-reproductive tumour types to date.[51,52]

Immunity and cancer

Influenced by sex hormones, sex chromosomes also have an impact on cancer immune defences.[53] In general, females are able to mount stronger innate and adaptive immune responses than males, which results in faster clearance of pathogens and greater vaccine efficacy in females than in males, but also explains the greater incidence of inflammatory and autoimmune diseases in females.[54,55]

Approximately 50 X-linked genes are involved in adaptive and innate immunity.[56] These X-linked genes code for proteins including pattern recognition receptors [such as Toll-like receptor 7 (TLR7) and TLR8], cytokine receptors [e.g. Interleukin 2 receptor subunit gamma (IL2RG) and Interleukin 13 receptor subunit alpha 2 (IL13RA2)], and transcriptional factors (e.g. FOXP3). The X-linked Forkhead box P3 (FOXP3), expressed in regulatory T (Treg) cells, is critical for immune homeostasis, as it limits the adaptive immune responses. In comparison with female visceral adipose tissue (VAT), male VAT is enriched in FOXP3+ Treg cells and presents a distinct molecular profile that is enforced by a sex hormone-dependent niche.[57] The X-linked TLR8 also performs a central role in Treg cell biology. Activation of TLR8 in Treg cells triggers selective inhibition of glucose uptake leading to their senescence, relieving Treg cell inhibition of effector T cells. In the case of the *TLR7* gene, TLR7 may escape XCI in immune cells, leading to enhanced TLR7 expression owing to biallelism in XX cells, which contributes to the higher risk of developing autoimmune disorders in women and in men with Klinefelter syndrome.[58] Concerning lymphoid cell subsets, adult females present a higher frequency of B cells during adulthood, whereas higher CD4+ T-cell counts, higher CD4/CD8 ratios, and lower presence of CD8+ T cells are reported throughout life, compared with age-matched males.[59-62]

Immune defence against cancer declines with age and shows sexual dimorphisms. Reduction in T-cell numbers occurs in both sexes with age, but a disproportionate decrease in T-cell and B-cell populations is more evident in older males. Two waves of epigenetic regulation depleting immune cell functions have been identified, the first one in the late 30s, with a similar impact across the sexes. Genomic differences between sexes increase after the age of 65 years, with a second wave in males in their early 60s that results in increased proinflammatory activity and innate immunity and lower adaptive immunity. This is delayed by 5-6 years in females, who exhibit greater adaptive immunity.[63]

A pan-cancer analysis to evaluate the sex-based variance of different genomic immune-related factors using The Cancer Genome Atlas showed differences in tumour mutation burden, neoantigen burden, tumour purity, cytolytic activity, CD8+ T cell, and expressions of immune checkpoint genes according to sex.[64]

Sex hormones and cancer

Sex hormones exert pleiotropic functions on multiple tissues. The estrogen effect depends on the activation of the estrogen receptor-α (ERα) and -β (ERβ). Activation of ERα promotes expansion and mobilization of haematopoietic stem cells, favours skin wound healing, promotes angiogenesis and endothelial cell precursor mobilization, reduces hepatocyte proliferation, and restrains the inflammatory role of macrophages. ERβ also blocks macrophage activation and, contrary to ERα, negatively regulates vessel formation. The androgen receptor promotes angiogenesis, liver cell proliferation, and macrophage activation through the stimulation of tumour necrosis factor and CC-chemokine ligand 4, while it also suppresses wound healing. Hormone pathways are interrelated, since androgenic hormones are converted into estrogens through the action of aromatase, resulting in indirect control of pathways affected by ERα.

In the tumour microenvironment, cancer cells secrete vascular endothelial growth factor (VEGF) A and platelet-activating factor in response to estrogens, enhancing proliferation and migration.[65,66] In addition, estrogens promote mobilization of bone marrow-derived precursors to cancer

stroma in breast tumours.[67] Sex hormones are partially responsible for the sex-related differences in immune response as ERα and ERβ are expressed by many types of immune cells, including T cells, B cells, dendritic cells, macrophages, neutrophils, and natural killer (NK) cells[68] and these receptors present differential expression among immune cell subsets, as ERα is up-regulated in T cells while ERβ is highly expressed in B cells.[69] Finally, age-related changes in sex steroid concentrations and sex steroid receptor signalling might subsequently contribute to age-associated changes of immune function and populations.[70]

SEXUAL DIMORPHISMS IN CRC TUMOUR BIOLOGY

The tumour biology of CRC has been proven to be different in males and females as a result of the sex hormones and sex chromosomes that can influence immunity.[71] In addition, key proliferative pathways in CRC tumourigenesis are regulated through estrogens. Estrogens have been reported to control the activity of a class of Kv channels (KCNQ1 : KCNE3), which regulate fundamental ion transport functions of the colon and ultimately promote cell proliferation and epithelial—mesenchymal transition through bi-directional interactions with the Wnt/β-catenin signalling pathway. At the same time, estrogen modulates proliferative responses to hypoxia via the novel membrane estrogen receptor G protein-coupled estrogen receptor (GPER), as well as by Hypoxia-inducible factor 1-alpha (HIF1A) and VEGF signalling. Differences in oncogene expression, such as a higher frequency of mutations of *STK11* in males, and sexual dimorphisms in proteomes of CRC cells may contribute to the disparities in tumour biology according to sex in these tumours.[72-74]

Lastly, sexual dimorphisms in the tumour microenvironment of colorectal tumours have been investigated using tissue microarrays comprising primary tumour, tumour-infiltrated lymph nodes, and uninvolved colon.[75] Differential gene expression was observed in pathways related to Treg function, T-cell activity, and T-cell exhaustion, amongst several others, in females compared to males.

SEXUAL DIMORPHISMS IN CLINICOPATHOLOGICAL FEATURES OF CRC

Whilst globally females diagnosed with CRC have better overall survival compared with males,[1,76] in some countries the 5-year survival rate among women has been reported to be lower than among men, especially after the age of 70 years.[77] The proportion of patients presenting right-sided tumours is higher among females than males, as it is the proportion of *BRAF*-mutated tumours. Right-sided colon tumours are often at a more advanced stage at diagnosis and are less differentiated, which might partially explain this lower 5-year survival rate in females.[78]

Sex hormones may explain the higher frequency of right-sided CRC in females. It has been proposed that exposure to estrogen is protective against the development of tumours with microsatellites instability (MSI), while the lack of estrogen in older females might increase the risk of MSI-high CRC.[79] *PIK3CA* mutations, associated with poorer prognosis, and methylation of CpG island in the 5' region of the $p16^{INKa}$ tumour suppressor also occur at a higher frequency in females.[80,81]

Sex-associated differences in the microbiome have also been reported in healthy individuals during their life span, some of which are mediated by sex hormones and conditioning the estrobolome (a gene repertoire of intestinal microbiota able to carry out estrogen metabolism).[82,83] Plus, the microbiome is highly conditioned by the exposome, which might also vary according to gender. In patients with CRC, sexual dimorphism of microbiome has also been reported.[84] The microbiome might be more stable in the male gut than in the female gut. The male gut shows an enrichment of rare species that may contribute to the stability of microbial communities, whereas in the female gut there is a loss of species that could be responsible for the vulnerable microbial communities with the development of CRC.

SEX-RELATED DIFFERENCES IN TREATMENT EFFICACY AND TOXICITY IN CRC

Chemotherapy agents

Fluoropyrimidines are the backbone of chemotherapy in CRC management. Dosage is based on body surface area but does not take into account sex differences. However, a substantial body of literature indicates sex-associated differences in pharmacokinetics of 5-fluorouracil (5-FU).[85] Plasma clearance, plasma clearance per kilogram, and dose have been found to be lower in females compared with males, whereas plasma life and area under the plasma concentration—time curve might be higher in females. On the contrary, volume of distribution, volume of distribution per kilogram, and dose per kilogram do not differ significantly between sexes. These differences may have an impact on outcomes and toxicity.

Toxicity associated with 5-FU has been reported to be more extensive for females than for males in terms of the number of different types of toxicity, maximum toxicity grade, and incidence of severe toxicities, including haematologic toxicities such as leukopaenia, neutropaenia, and thrombocytopaenia, and self-reported toxicity such as stomatitis, diarrhoea, nausea, vomiting, or hand-foot syndrome.[86-88] This increased toxicity might be due to lower clearance of 5-FU leading to higher plasma levels in females compared to males, as previously reported.[89] Concerning patient-reported outcomes, an investigation exploring the occurrence and severity of self-reported physical and psychological co-occurring symptoms in patients with stage IV CRC receiving different 5-FU-based chemotherapy schemes reported more severe worrying, lack of energy, and nausea in women.[90]

Similar data about toxicity have been reported with the use of capecitabine. In a cohort of 299 patients (163 males, 136 females) receiving capecitabine in the adjuvant setting, females had significantly higher dose-limiting toxicity than men. Incidence of all common toxicities was higher in

Table 2. Summary of the investigations exploring the influence of sex in the management of CRC

Treatment of investigation	Type of treatment	Author	Study population	*N* (male/female)	Aim of the study	Conclusions
5-FU	Chemotherapy	Chansky et al.[86]	Adjuvant setting (SWOG-8572, SWOG-8591) and advanced CRC (SWOG-8611, SWOG-8905)	505 (260/245)	Toxicity	Greater number of different types of toxicity, higher maximum grade toxicity, and higher incidence of severe toxicity (≥ grade 3) in females
5-FU	Chemotherapy	Sloan et al.[87,88]	NCCTG trials between 1980 and 1995 in the setting of adjuvant and advanced CRC	2448 (1355/1093)	Toxicity	Higher rates of toxicity in females (stomatitis, leukopaenia, alopecia, nausea, vomiting, and diarrhoea)
5-FU-based regimens	Chemotherapy	Mueller et al.[89]	Patients with CRC, pancreatic or cholangiocellular cancer	31 (31/10)	Elimination of 5-FU	Lower clearance of 5-FU in females
5-FU	Chemotherapy	Röhrl et al.[90]	Curative chemotherapy (neoadjuvant or adjuvant) and palliative chemotherapy for CRC	120 (73/47)	Patient-reported outcomes	More severe worrying, lack of energy, and nausea in females
Capecitabine	Chemotherapy	Ilich et al.[91]	Adjuvant setting CRC	299 (163/136)	Toxicity	Higher incidence of all common toxicities and of dose-limiting toxicity in females. Higher frequency of dose reduction and lower dose intensity in females
Fluoropyrimidine-based regimens	Chemotherapy	Wagner et al.[92]	ACCENT database	34 640 (18 664/15 976)	Toxicity	Higher incidence of ≥ grade 3 non-haematological (nausea, vomiting, stomatitis, diarrhoea, peripheral neuropathy and transaminitis) and haematological toxicities (neutropaenia and leukopaenia) in females
TAS102 + bevacizumab versus capecitabine + bevacizumab	Chemotherapy + targeted therapy	André et al.[93]	Frontline mCRC, not candidates for intensive chemotherapy (SOLSTICE clinical trial)	856 (466/390)	Subgroup analysis	Greater efficacy of TAS102 + bevacizumab in males (versus males treated with capecitabine + bevacizumab)
FOLFOX + panitumumab	Chemotherapy + targeted therapy	Raimondi et al.[94]	Frontline *RAS* wild-type mCRC	229 (152/77)	Efficacy and toxicity	No differences in ORR, OS, PFS, or clinical benefit. Higher rate severe toxicity, G3-4 thrombocytopaenia, any grade and G3-4 neutropaenia, and any grade conjunctivitis in females. Higher incidence of any grade skin rash and hypomagnesaemia in males
FOLFOXIRI + bevacizumab versus FOLFOX/FOLFIRI + bevacizumab	Chemotherapy + targeted therapy	Marmorino et al.[95]	Frontline mCRC (TRIBE and TRIBE2 clinical trials)	1187 (693/494)	Efficacy and toxicity	No differences in ORR or PFS. Any grade and G3-4 chemotherapy-related adverse events more frequent in females. Any grade nausea and vomiting and G3-4 nausea and vomiting more frequent in females. No differences in bevacizumab-related adverse events
Regorafenib	Targeted therapy	Kawakami et al.[97]	Refractory mCRC	96 (46/50)	Adherence to treatment	Higher rates of toxicity in females, associated with lower adherence to regorafenib
Regorafenib	Targeted therapy	Vandeputte et al.[98]	Refractory mCRC	136 (78/58)	Toxicity	More frequent G3-4 toxicity in females. Higher rates of fatigue, anorexia, hypertension, and rash in females
Encorafenib + cetuximab ± binimetinib	Targeted therapy	Ros-Montana et al.[99]	Previously treated *BRAF*-mutant CRC	59 (23/36)	Efficacy (OS and PFS) and toxicity	No statistical differences in efficacy (although greater clinical benefit in females with the triplet). Higher rates of toxicity in females, requiring dose modifications more frequently
Immune checkpoint inhibitors	Immunotherapy	Ye et al. *and Samstein et al.*[102,103]	mCRC (microsatellite status not specified)	99 (54/45)	Efficacy	Better OS in males (HR = 0.53, $P = 0.041$)

5-FU, 5-fluorouracil; G, grade; HR, hazard ratio; mCRC, metastatic colorectal cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

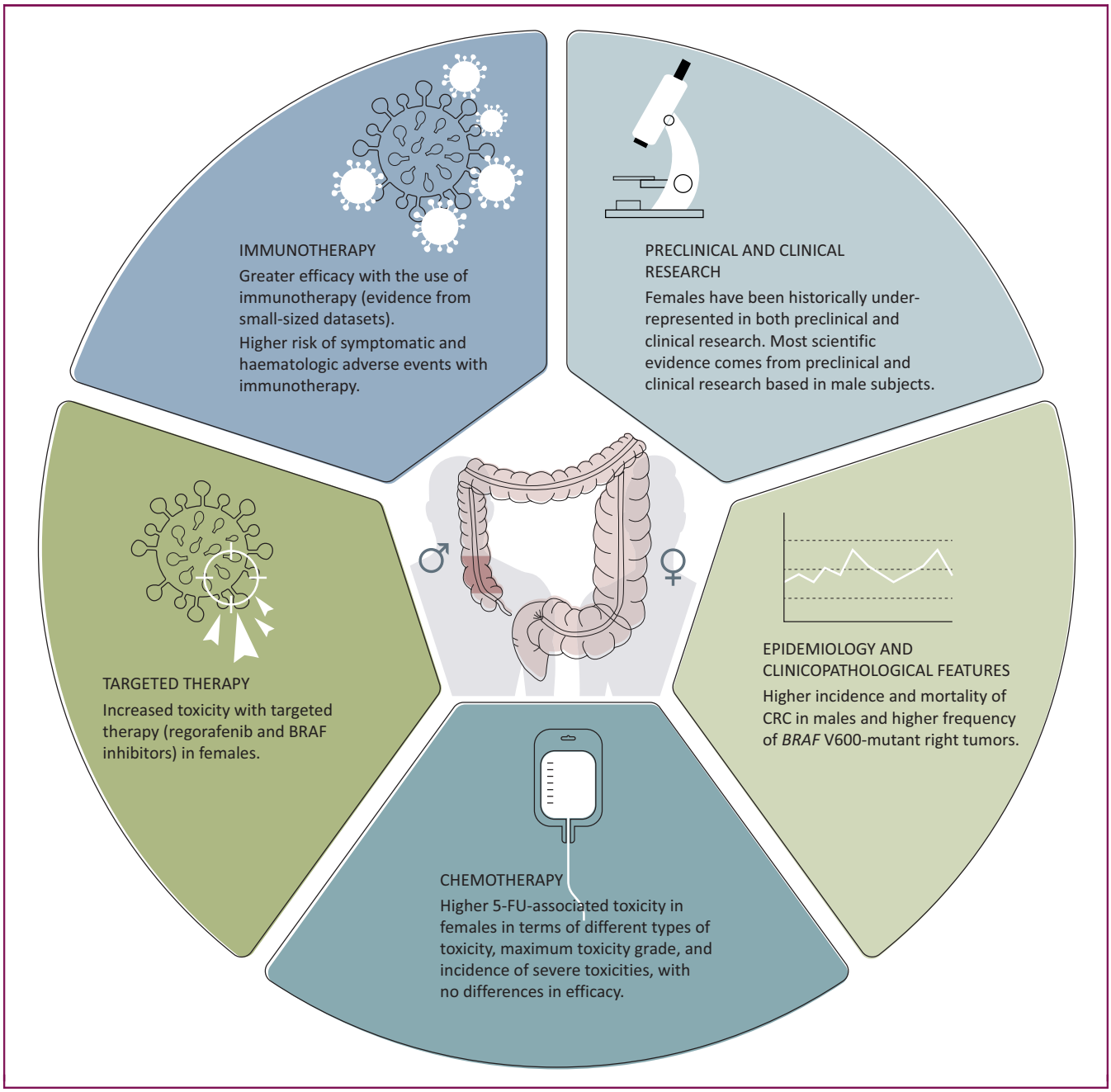

Figure 1. Sex and gender differences in colorectal cancer (CRC).
5-FU, 5-fluorouracil.

females and required significantly more dose reductions than males, leading to statistically significant lower relative dose intensities in females.[91] Consistent with these results, an analysis of 36 640 patients with colon cancer receiving adjuvant fluoropyrimidine-based chemotherapy in the ACCENT database showed that incidence of grade 3 or grade 4 non-haematological toxicity (nausea, vomiting, stomatitis, diarrhoea, peripheral neuropathy, and transaminitis) and haematological toxicity (neutropaenia and leukopaenia) was statistically significant and clinically relevant higher in females.[92]

Differential efficacy according to sex has only recently been studied. The SOLSTICE trial, comparing standard capecitabine and bevacizumab versus TAS102 and bevacizumab as frontline therapy for patients with metastatic CRC (mCRC) not candidates for intensive chemotherapy, did not meet its primary point in terms of progression-free survival (PFS). But interestingly, statistical differences for PFS were found in the subgroup analyses for males treated with the experimental treatment in comparison with those treated with capecitabine and bevacizumab.[93] A subgroup analysis of the VALENTINO trial, a multicentre, randomized, phase II trial, investigating two panitumumab-based maintenance strategies following first-line panitumumab plus FOLFOX in *RAS* wild-type mCRC patients, showed no significant differences in PFS, overall survival, overall response rate, or clinical benefit rate according to sex, but a significantly higher rate of grade 3-4

toxicity in females, compared to males.[94] Similarly, in the phase III trials TRIBE and TRIBE2 investigating first-line FOLFOXIRI/bevacizumab or a doublet (FOLFIRI or FOLFOX)/bevacizumab, no statistical differences were reported depending on age or sex, although an increased risk of grade 3-4 toxicity was found in elderly females.[95] However, it must be noted that these studies aimed to explore overall differences between sexes but were not specifically designed to compare efficacy of each arm between males and females.

These results suggest that patient sex should be taken into account and that conventional methods of using body surface area for dosing may be inaccurate. Therefore, therapeutic drug monitoring has been proposed as an alternative dosage of 5-FU as it might lead to therapeutic plasma levels with a maximized risk/benefit ratio.[96]

Targeted therapies

The use of targeted therapies for patients with CRC has been limited to antiangiogenic agents or epidermal growth factor receptor inhibitors in combination with chemotherapy for years. The impact of sex and gender on efficacy and toxicity of these therapies might therefore be obscured by the concomitant use of chemotherapy. Regorafenib, a multi-kinase inhibitor, obtained approval for refractory mCRC in monotherapy. More frequent toxicity in terms of fatigue, anorexia, hypertension, and rash, as well as severe toxicity have been reported in females compared with males, which might lead to lesser adherence to treatment.[97,98]

Only recently, the use of BRAF inhibitors without chemotherapy has proved efficient in patients with mCRC harbouring *BRAF* V600 mutations. Influence of sex on efficacy and toxicity with the use of BRAF targeted therapy has been reported with the use of encorafenib plus cetuximab, with or without binimetinib. Among patients with mCRC harbouring *BRAF* V600E mutations treated with these combinations, a trend for superior clinical benefit in females, particularly with the triplet combination but with a higher toxicity cost, was observed.[99]

Ongoing trials testing the efficacy of targeted therapies, such as KRAS G12C inhibitors or anti-human epidermal growth factor receptor 2 (HER2) therapies, should specifically address these sex-associated differences.

Immunotherapy

Efficacy of immunotherapy according to sex across different tumour types has been investigated with inconsistent conclusions.[100,101] Given that the use of immunotherapy in CRC is limited to mCRC presenting MSI, analyses exploring this phenomenon usually include only a small number of patients. Better overall survival has been reported in males compared with females in patients with mCRC treated with immune checkpoint inhibitors.[102] However, this analysis included only 99 patients, 45 of whom were females and with an uncertain number of patients with MSI tumours.[103]

In an analysis exploring symptomatic and objective toxicities across multiple cancer types, including gastrointestinal tumours, and patients treated with immunotherapy, the risk of symptomatic and haematologic adverse events was higher for females receiving immunotherapy compared with males, while the risk of objective non-haematologic toxicity was similar for both groups.[104] Concerning the mechanism of action of immunotherapy, females receiving immune checkpoint inhibitors and immune system modulators had a higher risk of symptomatic toxicity, but this association was not observed for asymptomatic toxicity.

Taken together, these data support designing trials addressing specifically sex and gender, as the balance between efficacy and toxicity may be improved by the development of sex-specific dosing strategies (Table 2, Figure 1).

CONCLUSIONS

The consequence of failing to include sex-based differences in study design and analyses has repeatedly led to 'one-drug' treatment regimens for both males and females.The impact of gender on health outcomes, which are different from those influenced by biological sex, is even more unknown, since gender variables are seldom included in patients' medical histories. In the literature, these terms are often used interchangeably, but the results of the investigations that have been achieved so far exploring the sexual differences are mainly focused on sex indeed. This is due to lack of information about gender, that, because of its nature, must be specifically obtained by directly questioning the patient. In this sense, professionals should be trained in the importance of data collection about gender identity as a first step to be able to move forward in this field.

Precision oncology is not limited to exploring molecular biomarkers and it requires deeper understanding of biological sex and gender differences. If the long-term goal of personalizing treatments for patients with CRC is effective treatment for all individuals, then the sex and gender must be accounted for. Despite the calls from the NIH, FDA, industry, and advocacy, the path ahead is long. Since basic science and translational research serves as the cornerstone for clinical research and medical decision making, sex disparities in physiology and pathophysiology cannot be neglected.

In our perspective, barriers to enrolment of females in clinical trials in oncology should be addressed through partnership with all stakeholders, including patients, investigators, referring clinicians, health care systems, and social community. Interventional clinical trials with the focus on investigating sex-specific differences in efficacy and toxicity (including both objective toxicity and measurement of patient-reported outcomes) as a primary endpoint and the evaluation of specific dosing regimens according to sex are needed to improve outcomes. In endorsing research on how biological sex and gender influence CRC, we have the opportunity for greater precision, as it might usher in the

development of sex-specific therapeutics with greater efficacy and safer toxicity profile for our patients.

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