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Toren, Paul; Wilkins, Anna; Patel, Keval; Burley, Amy; Gris, Typhaine; Kockelbergh, Roger; Lodhi, Taha; Choudhury, Ananya; Bryan, Richard T

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THE SEX GAP IN BLADDER CANCER SURVIVAL - A MISSING LINK IN BLADDER CANCER CARE?

Paul Toren[1], Anna Wilkins[2,3], Keval Patel[4], Amy Burley[2], Typhaine Gris[1], Roger Kockelbergh[5,6], Taha Lodhi[7], Ananya Choudhury[7], Richard T Bryan[6,8].

Affiliations:

1. CHU de Québec-Université Laval, Quebec City, Canada.
2. Division of Radiotherapy and Imaging, The Institute of Cancer Research, London, UK.
3. The Royal Marsden Hospitals NHS Trust, London, UK.
4. University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.
5. University Hospitals of Leicester NHS Trust, Leicester, UK.
6. Action Bladder Cancer UK, Tetbury, UK.
7. Division of Cancer Sciences, University of Manchester and The Christie NHS Foundation Trust, Manchester, UK.
8. Bladder Cancer Research Centre, Institute of Cancer & Genomic Sciences, University of Birmingham, UK.

Correspondence:

Professor Richard T Bryan

Bladder Cancer Research Centre, Institute of Cancer & Genomic Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

r.t.bryan@bham.ac.uk

ORCIDs

Paul Toren: 0000-0002-5762-5787

Anna Wilkins: 0000-0002-0425-584X

Keval Patel: 0000-0003-1913-0852

Amy Burley: 0000-0003-3074-6439

Typhaine Gris: TBC

Roger Kockelbergh: 0000-0003-2261-3628

Taha Lodhi: 0000-0002-6891-0866

Ananya Choudhury: 0000-0002-3561-6580

Richard Bryan: 0000-0003-2853-4293.

ABSTRACT

Recent data again bring to light again differences in bladder cancer outcomes between sexes. Uncommon among cancers, bladder cancer outcomes are notably worse for women than for men. Furthermore, bladder cancer is 3-4 times more common amongst men. In this perspective, we review the current understanding of factors which may explain these sex differences. These include understanding the importance of haematuria as a symptom of bladder cancer by both clinicians and patients, the resultant delays in diagnosis and referral of female patients with haematuria, and healthcare access. Notably, these factors appear to have geographical variation and are not consistent across all healthcare systems. Likewise, there are inconsistent data relating to sex-specific treatment responses for both NMIBC and MIBC patients. The impact of differences in the microbiome, bladder wall thickness and urine dwell times remain to be elucidated. The interplay of hormone signalling, gene expression, immunology and tumour microenvironment remains complex but likely underpins the sexual dimorphism in disease incidence and stage and histology at presentation. The contribution of these biological phenomena to sex-specific outcome differences is probable, albeit potentially treatment-specific, and further understanding is required. Notwithstanding, we identify opportunities to harness biological differences to improve treatment outcomes, as well as areas of fundamental and translational research to pursue. At the level of policy and healthcare delivery, improvements can be made across the domains of patient awareness, clinician education, referral pathways, and guideline-based care. Together, we aim to highlight opportunities to close the sex gap in bladder cancer outcomes.

INTRODUCTION

Bladder cancer (BC) is the tenth most common cancer worldwide, responsible for 3% of annual cancer diagnoses and 2.1% of cancer-related deaths[1]. Most patients (75-80%) present with non-muscle-invasive bladder cancer (NMIBC: stages Ta/T1/Tis) – up to 80% of these patients will experience recurrence[2], and up to 44% will progress to muscle-invasive bladder cancer (MIBC: stages T2-4)[2-4]. Moreover, of the 20-25% of patients initially diagnosed with MIBC, around one-quarter will have incurable, locally-advanced or metastatic disease[5]. Muscle-invasion thus represents a critical step in the disease course, carrying a 5-year survival of only 27-50%, despite radical therapies[3].

Overall, the number of incident BC cases continues to increase, although the age-standardized incidence and mortality appear to be decreasing, paralleled by fewer smoking-related cases[6]. Smoking is the largest modifiable risk factor for BC, with exposure to occupational carcinogens also well documented[7]. However, sex is the largest risk factor for BC, with men 3-4 times more likely to be diagnosed with BC than women[8,9]. Furthermore, there is no clear indication of the differential effects of smoking between men and women[10]. Nonetheless, women tend to have more aggressive tumours at diagnosis and experience worse outcomes thereafter[11-15].

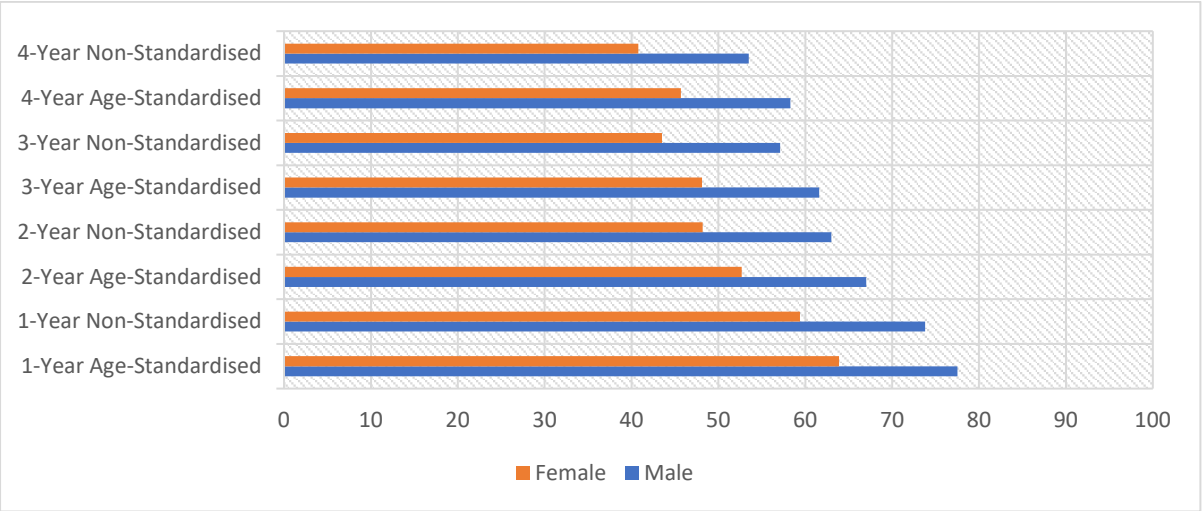
BC cases which are muscle-invasive or metastatic at presentation contribute the most significant morbidity and mortality, while the surveillance of NMIBC drives BC to be one of the most expensive per patient cancers to treat[16]. Therefore, there is a need to identify and fill the sex-based gaps in our understanding and care for all BC patients. In this perspective, we review the differences in outcomes between men and women with BC, discuss our current understanding of these differences, and propose solutions to pursue. These differences are significant given the impact of BC on patients and healthcare systems.

SEX DIFFERENCES IN OUTCOMES

Recent data regarding cancer survival in England (cancers diagnosed from 2015 to 2019, followed up to 2020[17]) appear to lay bare the persistent sex gap in survival for BC patients. Across all stages of BC and for 1-, 2-, 3- and 4-year BC survival, female patients had worse outcomes than male patients (**Figure 1**). With some 3- and 4-year stage-specific survival data missing for female patients (only), a knowledge deficit may already be apparent. Notwithstanding, using an overlapping dataset available for T1–T4 tumours treated from 2013 to 2019, recent analyses of overall survival for urothelial cancer patients in England by Catto et al also illustrate a similar situation[18]. Beyond the UK, such gaps in outcomes continue to be highlighted internationally[19-23].

Figure 1: *1-year, 2-year, 3-year, and 4-year bladder cancer survival (%) for adults (aged 15 to 99 years) diagnosed in England 2015 to 2019, followed up to 2020[17]. **(a)** Age-standardised and non-age standardised net survival (%) by sex. **(b)** Age-standardised net survival (%) by stage and sex. **NB:** 3-Year Stage 1, 3-Year Stage 4, 4-Year Stage 1, 4-Year Stage 3 and 4-Year Stage 4 data are all missing for female patients. Age-standardisation represents a weighted-average of mortality rates for each sex based on the International Classification of Survival Standard.*

(a)



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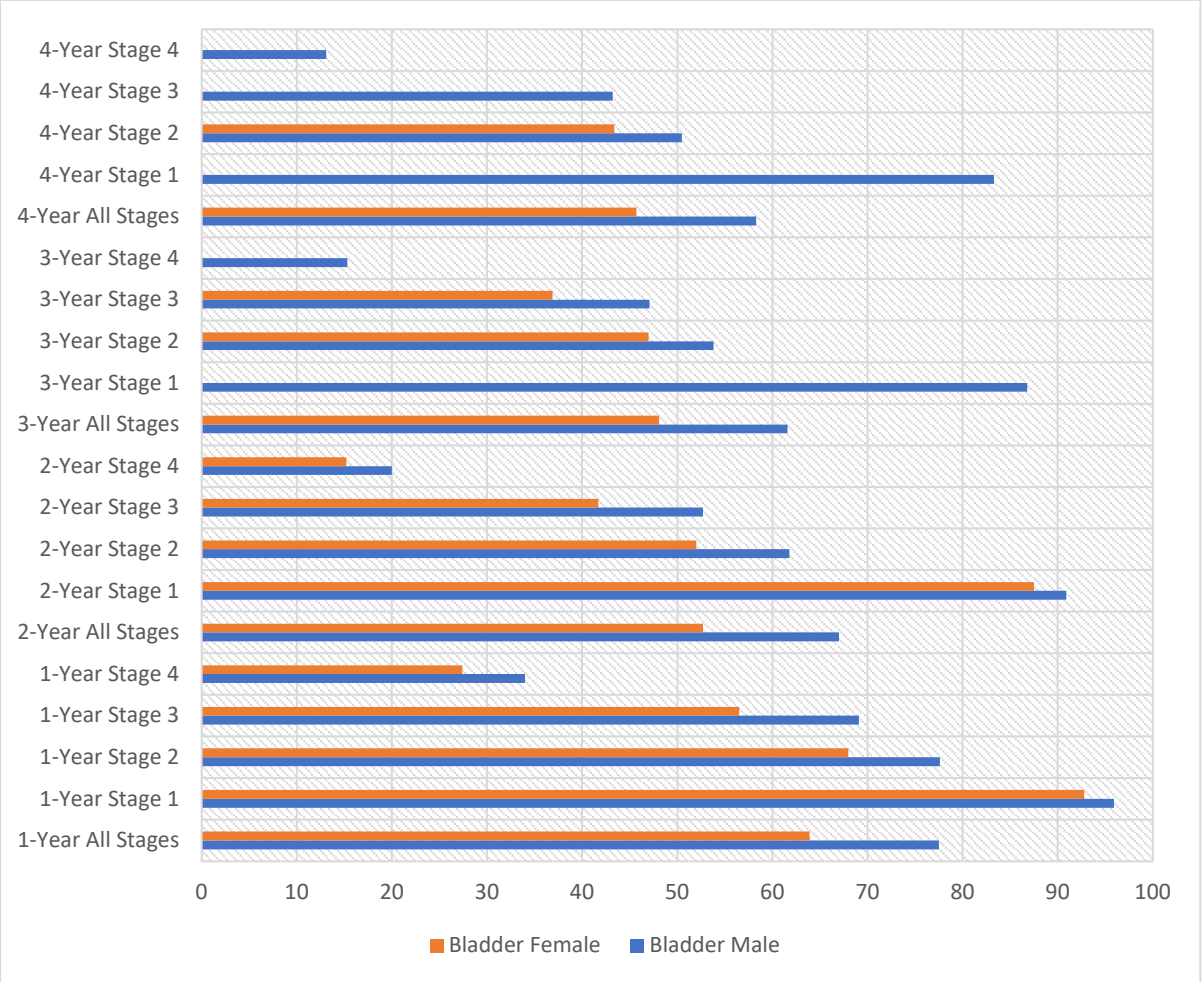
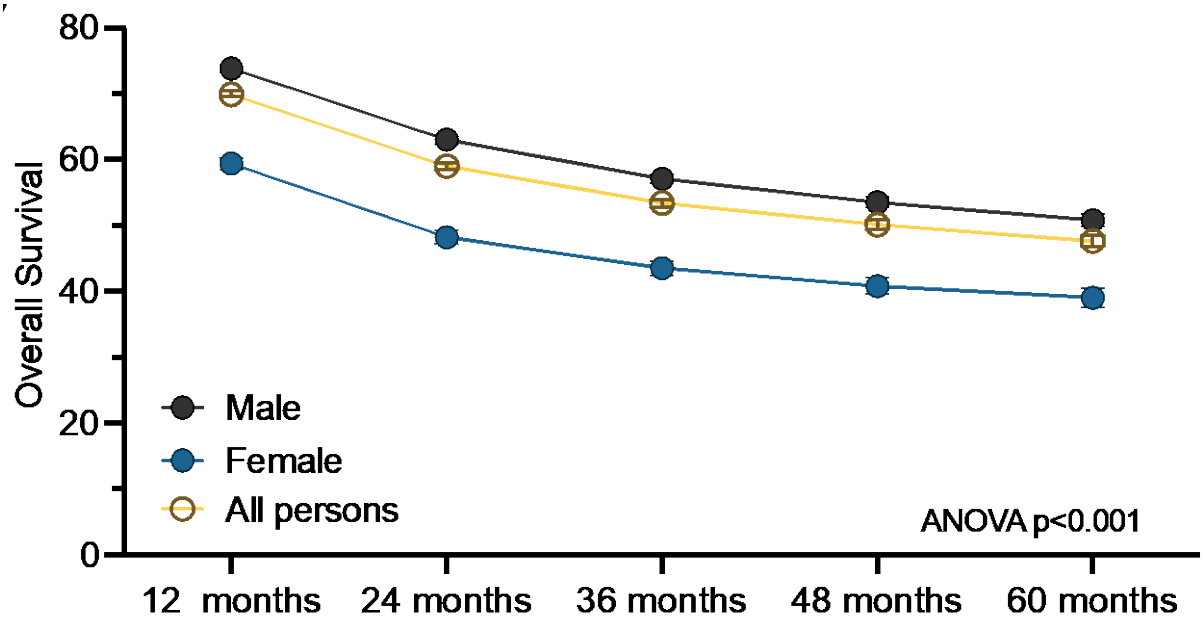


Figure 2: *Overall survival for patients with T1–T4 bladder cancers treated in England from 2013 to 2019, plotted using the Kaplan-Meier method with respect to sex (reproduced with kind permission from John Wiley and Sons[18]).*



This is neither a new phenomenon, nor an unexplored phenomenon[19,22], yet it remains unexplained worldwide. Moreover, these findings in BC are the inverse of the situation for most cancer sites where cancer outcomes are commonly worse for men[24]. Previous European data from 1999-2007 also reported lower BC survival among women, driven by differences in Northern and Central European countries and the UK[25]. More recent data from the Netherlands also mirrors the UK data[26]. However, other studies have found no differences in treatment or cancer-specific survival[27]. A 2022 systematic review and meta-analysis concluded that female sex was associated with worse cancer-specific survival and overall survival in patients with MIBC, but no differences were apparent in the relatively lower number of studies with sex-specific outcomes for NMIBC[28]. Concordant with the recent NHS results, this suggests that sex differences in survival are principally driven by patients with non-metastatic MIBC at presentation, or those who progress to develop MIBC.

POSSIBLE REASONS FOR DIFFERENCES IN OUTCOMES

The possible explanations are complex – the interplay of the signs and symptoms of the disease, speed of referral into the BC diagnostic pathway, treatment decisions, treatment responses, and biology[29]. It is important to better understand the weight and relevance of each of these factors in order to take concrete steps to further investigate and potentially close the apparent gap in overall outcomes.

Recognising the importance of haematuria

Female patients may not recognise the importance of haematuria in a disease where there is a 3:1 male preponderance or may not seek investigation as early[21]. Haematuria is the most common symptom of bladder cancer[2]. However, from a young age, women are much more likely to present with a UTI and have associated haematuria in some instances. This can desensitize both patients and clinicians to not recognize the importance of haematuria, particularly in older women at higher risk of bladder cancer[30].

Referral for investigations

Once haematuria has been identified as a potential symptom of bladder cancer, there is a need for prompt referral according to guidelines. The preponderance of haematuria related to urinary tract infection in women can result in delays in diagnosis – as reported internationally, female patients presenting with haematuria to their primary care provider are not referred for subsequent investigations as rapidly as male patients or are investigated less thoroughly[21,29-34]. Indeed, Lyratzopoulos *et al.* found that women had a significantly higher number of pre-referral consultations than men when presenting with haematuria, with 27% of women requiring 3 or more consultations compared with only 11% of men requiring the same[31].

Treatment decisions

In the setting of NMIBC, sex does not appear to influence the utilisation of adjuvant intravesical therapy[35], nor the choice of radical treatment for MIBC[36-38] and, in terms of overall treatment paradigms, there are currently no differing recommendations between sexes. Although the use of continent urinary diversions appears to be lower in female patients than male patients[39,40], this does not alter survival outcomes. This may reflect higher rates of advanced tumour stage in women, but also potential differences in training and practice patterns, with fewer centres worldwide equally comfortable to perform orthotopic continent diversion in women given anatomical and surgical differences pertinent to differences in tumour stage. However, for female patients undergoing radical cystectomy and bladder substitution, preservation of the uterus and attempted nerve-sparing appear to result in better functional outcomes[41], yet there remain significant gaps in the adoption of female reproductive organ-sparing and nerve-sparing radical cystectomy techniques for patients with organ-confined disease[42]. With emerging data from large series confirming the low rate of female reproductive organ involvement at the time of radical cystectomy (4.2-5.7%)[43,44], there appear to be potential sex disparities driven by provider expertise and preference. These disparities may need to be addressed through training and/or the refinement or centralisation of specific provider expertise.

Treatment efficacy

Evaluating whether female patients derive less benefit from current treatments is more difficult to dissect and may be, in part, driven by biological and anatomical reasons elucidated in the section below. Female patients nonetheless present with worse disease stage[9,33,38,45], and more often with non-urothelial tumour histology[9,20,46], thus contextualising subsequent sex-specific treatment responses. Studying 24,169 BC patients in The Netherlands, Richters *et al.* found that, in the first two years after diagnosis, excess mortality rates for women were higher than for men but lower thereafter[9]. This applied to both NMIBC and MIBC patients, and baseline differences in age, stage, and histology accounted for only part of the excess mortality gap[9]. Ballas *et al.* reported similar findings for patients undergoing bladder preservation (trimodality therapy) for T2-T4a N0 M0 MIBC[47]. Marinaro *et al.* studied 47,229 MIBC patients in the USA and identified increased 90-day mortality following radical cystectomy and worse overall survival in female patients[37]. Others have also highlighted either worse cancer-specific survival in females than males following radical cystectomy[36,45], or both worse cancer specific and overall survival[11,48]. Notwithstanding, other research suggests that higher uptake of neoadjuvant chemotherapy diminishes these sex differences[49]. Such data may indicate that if sex differences in outcomes are attenuated in patients fit enough for chemotherapy then, simply by selection bias, similar outcomes could also be expected in the generally fitter patients enrolled into clinical trials. In the radiotherapy setting, unpublished data from Manchester, UK, regarding 209 MIBC patients treated by radiotherapy with concurrent carbogen and nicotinamide (BCON protocol[50]) show no difference in 5-year cancer-specific survival between males and females.

In addition to overall survival data, conflicting data report on potential sex differences in response to specific treatments for BC[51]. Evaluating response to intravesical treatment for NMIBC, earlier reports (summarised in a 2018 meta-analysis and systematic review[52]) suggest women have poorer responses to BCG. However, there appears to be publication bias in this meta-analysis toward studies reporting a sex-based difference and, thus, there is doubt as to these conclusions suggesting differential responses in NMIBC to BCG[52]. In line with this, two large contemporary cohorts of BCG-treated NMIBC patients did not report any sex differences in outcomes of recurrence-free survival or progression-free survival[53,54]. Furthermore, the literature suggests no sex differences in response to intravesical chemotherapy for NMIBC.

For advanced BC, the accumulating evidence does not suggest that sex-based differences exist in disease response to immune checkpoint blockade (ICB); this includes the sex-based analyses presented in recent trials, including KEYNOTE-052[55], KEYNOTE-361[56], and IMvigor130[57]. Moreover, the best available data do not suggest sex-specific differences in response to chemotherapy, with Haines

et al. concluding that "female patients with metastatic urothelial cancer tolerate cisplatin-based chemotherapy similarly to male patients and achieve comparable clinical outcomes"[58]. Biological tumour differences and stage differences in presentation confound these studies, which together suggest that, stage-for-stage, treatment responses remain generally similar between men and women. Hence, there may be regional or treatment-specific circumstances where sex does not appear to be a prognostic factor.

Biological and anatomical phenomena

Few studies have thoroughly assessed the fundamental sex differences in urothelial transformation and subsequent cancer biology – the interplay of sex hormones, environmental exposures, microenvironment, microbiome, immunology, and genomics are important and highly complex[19,22]. Notwithstanding, the laboratory studies available to date provide some insights into potential drivers of biological differences in bladder tumours which may drive differences in outcomes between men and women.

<u>Sex hormones</u>

Knockout studies in mice suggest that the androgen receptor (AR) in the urothelium is important for urothelial carcinogenesis[59,60]. Further, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) knockout mice experiments suggest that ERα has a protective role against BC initiation and progression, while ERβ could promote BC[61-63]. These murine results are consistent with immunohistochemical studies demonstrating downregulated ERα expression in higher grade and higher stages bladder tumors[64] and upregulated ERβ and aromatase protein expression in higher grade and more aggressive bladder tumors[64-67]. Using the "four cores genotypes" mouse model, which decouples chromosomal and gonadal sex, researchers found gonadal sex (i.e. hormonal exposure) had the single largest impact on chemically-induced bladder tumour development[68]; notwithstanding, chromosomal sex also independently influenced tumour development[68].

Sex steroids have direct effects on the activity and function of various subsets of innate and adaptive immune cells and are known to contribute to immunological differences between sexes. The role of systemic hormones in the incidence and progression of bladder tumours remains incompletely defined, particularly in women. Epidemiologic studies of BC among women suggest a later age at menopause, parity (vs nulliparity) and use of hormone replacement therapy (HRT) may be associated with decreased BC incidence[69-73]. However, a meta-analysis suggested that the age of menarche does not affect the risk of BC in women[74], and so a need for clarification remains.

Genomics

The genomic and molecular understanding of BC has advanced in recent years[75-79]. MIBCs are heterogeneous[80] and characterised by many single nucleotide variants (SNVs) and copy number variants (CNVs)[75,81,82]; loss of multiple tumour suppressors and alteration of multiple pathways are common[77,78]. Six consensus gene expression-based subtypes of MIBC are now recognised and share some characteristics[77], but which remain heterogeneous with respect to genomic aberrations and behaviour; temporal and spatial plasticity in subtype has also been reported[83]. See **Table 1** below. NMIBC is arguably more complex than MIBC[78,79], comprising multiple grades of disease[84].

Within this landscape, BCs (alongside other cancers) demonstrate extensive sex-biased molecular signatures, with sex-biased expressed genes enriched in the sex chromosomes and evidence of sex-biased DNA methylation patterns (e.g. *TOP2B*)[85]. Sex-biased pathways include those related to immune responses, apoptosis and the cell cycle, metabolism, DNA repair and P53 pathways[85]. Furthermore, *KDM6A* alterations are common in BCs (24-33%[75,79]) with the gene functioning as an epigenetic regulator of downstream gene expression; importantly, *KDM6A* escapes X chromosome inactivation[19]. Mouse models appear to demonstrate that loss of *Kdm6a* increases BC risk in female mice, and mutations or reduced expression of human *KDM6A* predicts poor prognosis in female BC patients[68]. Other research has suggested a higher rate of *KDM6A* mutations in NMIBCs from female patients[86].

Table 1: Summary of the main characteristics of the consensus classes of MIBC (adapted from[77]).

Class name	*Luminal Papillary (LumP)*	*Luminal Non-Specified (LumNS)*	*Luminal Unstable (LumU)*	*Stroma-rich*	*Basal/Squamous (Ba/Sq)*	*Neuroendocrine-like (NE-like)*
% of MIBC	24%	8%	15%	15%	35%	3%
Oncogenic mechanisms	FGFR3+ PPARG+ CDKN2A-	PPARG+	PPARG+ E2F3+ ERBB2+ Genomic instability Cell cycle+		EGFR+	TP53- RB1- Cell cycle+
Mutations	*FGFR3* (40%) *KDM6A* (38%)	*ELF3* (35%)	*TP53* (76%) *ERCC2* (22%) TMB+ APOBEC+		*TP53* (61%) *RB1* (25%)	*TP53* (94%) *RB1* (39%)
Clinical characteristics	T2 stage +	Older patients + (80+)			Women + T3/T4 stage +	
Median overall survival (years)	4.0	1.8	2.9	3.8	1.2	1.0

Histopathology & molecular pathology

In BC pathology, there are several notable differences between sexes. A review of over 27,000 patients in the National Cancer Database in the US found women have more non-urothelial carcinoma (15.1% vs 9.9%), with squamous carcinoma the predominant histology[46]. Similarly, UK national data from over 100,000 T1-T4 BC patients demonstrated that women had more non-urothelial cancer than men (27% vs 16%, respectively)[87]. For MIBC, women appear to have a higher proportion of tumours with a basal molecular subtype, while men have a higher proportion of luminal papillary and neuro-endocrine-like subtypes[88]. However, an analysis of NMIBC did not identify differences in molecular subtypes according to sex[89].

Tumour immunology & microenvironment

The wide repertoire of immunomodulatory agents used across stages for treatment of BC, including in clinical trials, means that the contribution of sex-specific immunological and microenvironmental factors is likely to be clinically important. Such factors can include genetic, epigenetic and transcriptional effects[85], which may relate to XX and XY chromosomal differences[90], as well as hormonal effects[91]. Several pre-clinical and clinical studies highlight these factors and provide some explanation for the divergent observations of increased incidence of BC in male populations yet inferior survival outcomes in women described earlier.

Studies of bladder tumour AR expression do not show sex-based differences in expression[92]. However, Kwon *et al.* have recently described a fascinating androgen-driven mechanism of T cell exhaustion in BC[91]. Their insights provide some explanation for why spontaneous rejection of early immunogenic bladder tumours is less common in males, and hence the male predisposition for the development of BC. The study evaluates three different bladder tumour models, MB49 (transplantable syngeneic tumours), BBN (carcinogen-induced tumours) and BKL171 where BBN-induced tumours develop in a testis-bearing mouse with an XX chromosome to eliminate any immune response to male-specific minor antigens. The authors demonstrate more aggressive tumour growth in male versus female mice with these three models[91]. This effect was eliminated using Tcrb/Tcrd or *RAG2* knockout mice, which specifically lack T cells, and reinstated with adoptive transfer of $CD8^+$ T cells. A two-fold higher frequency of polyfunctional $CD8^+$ T cells able to produce Interferon gamma (IFNγ), Tumour Necrosis Factor Alpha (TNFα) and Granzyme B (Gzmb) was seen in MB49 tumours of female versus male mice at day 9 of tumour growth.

In support of the above observations, single-cell RNA sequencing identified increased effector-like $CD8^+$ T cells in the female versus male tumour microenvironment (TME). In contrast, the male TME was enriched for progenitor exhausted $CD8^+$ tumour infiltrating lymphocytes (TILs), as defined by their stem-like genetic profile (i.e. Tcf1/$Tcf7^+$). These TILs showed accelerated progression to terminally differentiated Tcf1-$Tim3^+$ exhausted T cells incapable of restimulation. In keeping with all of the above results, male mice with loss of AR exclusively in $CD8^+$ T cells were equally protected against cancer as female mice. Finally, the authors observe a negative correlation between type I interferon signalling and AR activity in T cells and they suggest this balance may underlie wider sexual dimorphism in cancer immunity. Insights from the above study are corroborated by observations elsewhere that androgen deprivation therapy can promote responses to ICB[92,93]. The work by Kwon *et al.* is $CD8^+$ T cell specific; $CD4^+$ T cells are known to be important in BC immunology[94], and there are likely further sex-specific mechanistic insights to T cell biology to be uncovered in the future.

Studies to explore a biologic basis for different responses to BC therapy have yielded interesting results. An analysis of the whole transcriptomes of 460 tumours from the UROMOL cohort, together with multiplex immunofluorescence of tumours from the Kingston Health Services Centre (KHSC) cohort (n=332, 22% female)[95] reported increased expression of the immune checkpoint genes *CTLA4*, *PDCD1*, *LAG3*, and *ICOS* in high-grade tumours from females compared to high-grade tumours from males or low-grade tumours from either sex. In addition, increased expression of CXC ligand 13 (*CXCL13*, an important B-cell–recruiting chemokine) and the B-cell surface-associated molecule *CD40* were seen more frequently in high-grade tumours from female patients. Intriguingly, the authors also observed increased infiltration of $CD163^+$ M2-like tumour-associated macrophages (TAMs) in both low- and high-grade NMIBC tumours of female patients versus those of male patients[95]. Of further clinical relevance, a higher density of $CD163^+$ M2-like TAMs and $CD79a^+$ B cells was independently associated with shorter recurrence-free survival across all high-grade tumours in the KHSC cohort supporting a functional relevance of this sexually dimorphic observation. Together, these findings suggest that the TME of NMIBC from female patients tends towards greater immune exhaustion where immune dysfunction is accentuated by reciprocal communication between increased immunosuppressive macrophage and B cell populations.

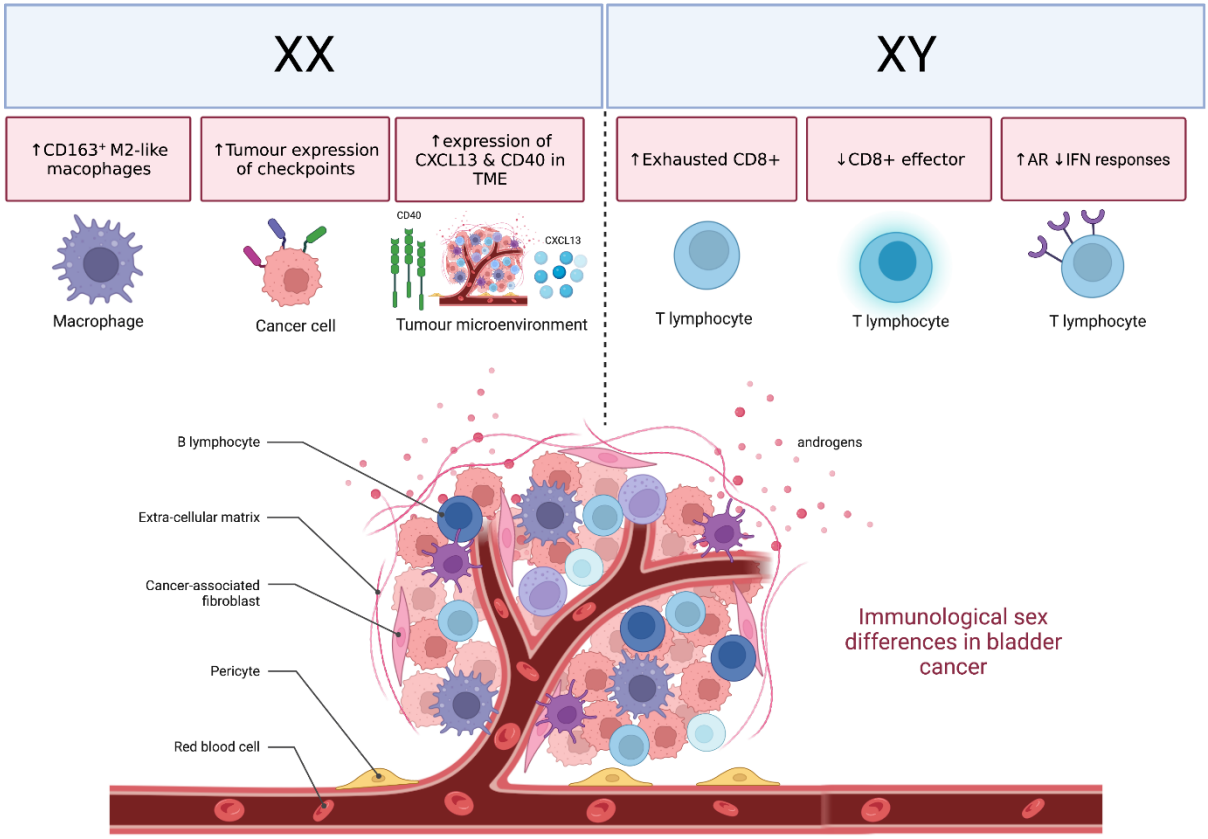
Elsewhere, research findings indicate that estrogen inhibits interleukin-6 and thus decreases the expression of receptor complexes required for BCG adherence to urothelial cells, such as integrin $\alpha5\beta1$[96,97]. A therapeutic strategy combining the anti-estrogen therapy ICI 182780 with BCG was shown to improve treatment efficacy in *in vitro* and *in vivo* pre-clinical systems, in part via enhancing TNFα signalling[98]. Furthermore, the immune checkpoint ICOS shows greater upregulation in whole blood following stimulation of healthy female volunteers versus their male counterparts with BCG[99]. However, these findings require careful interpretation in view of the considerable immunological differences between post-menopausal women and younger healthy volunteers. Overall, a current key research gap is to understand the sex-specific longitudinal innate and adaptive immune changes that occur both intra-tumourally and systemically over the months to years following BCG treatment, including how these relate to a differential therapeutic response.

Immunological ageing or immunosenesence is known to have sex-specific biological characteristics[100]. A recent study using bulk RNA sequencing showed an enrichment for B cell function-associated pathways in aged healthy female mice bladders versus their aged male counterparts[101]. Multiplex immunofluorescence confirmed a greater number of organised tertiary lymphoid structures (TLS) in the healthy bladders of female mice. Somewhat surprisingly, in murine bladders treated with several weeks of the carcinogen BBN, there was no difference in TLS between male and female mice. Instead, an

increase in plasma cells was seen in the lamina propria of female aged mice, and female mice had a more immune-infiltrated and oedematous lamina propria across ages.

As outlined earlier, a difference in the response to ICB according to sex has not been clearly established in BC. However, we know that immune cell PD-L1 is associated with inferior survival outcomes across sexes, and that androgen signalling in T cells represses IFNγ to limit ICB responses[93]. Furthermore, estrogen plus a number of X-linked micro-RNAs, including miR-221, miR-222, and miR-106b, can regulate PD-L1 expression[90,102]. Our incomplete understanding of the sex-specific immunogenomic changes underlying differential responses to ICB urgently warrants further research to optimise novel combination strategies across disease stages. See **Figure 3**.

Figure 3: *A summary of immunological sex differences in BC: The female TME is characterised by increased infiltration of immunosuppressive CD163+ M2-like macrophages and increased tumour expression of immune checkpoints. In addition, an increase in genes related to B cell recruitment (CXCL13) and function (CD40) has been shown in the female TME. In contrast, the male TME is characterised by increased exhausted CD8+ T cells (both of progenitor and terminally differentiated subtypes), which is driven by AR signalling specifically in CD8+ T cells.* ***AR****: androgen receptor,* ***IFN****: interferon,* ***TME****: tumour microenvironment.*



Microbiome

Current research seeks to define whether differences in the urinary, tumoral or gut microbiome between men and women may contribute to differential outcomes[103]. While urinary microbiome differences may be associated with the risk of recurrent urinary tract infections (UTIs) contributing to carcinogenesis, the causal implication of potential microbiome differences in tumour progression or treatment response remains to be defined[22].

Anatomy

Anatomically, men have more outlet obstruction related to prostatic enlargement and subsequent detrusor hypertrophy. Women have thinner bladder walls[104], which may help explain a higher incidence of non-organ-confined disease at diagnosis[45]. Differential urinary dwell times in men and women may contribute to differences in BC development, with men more commonly having higher post-void residuals with age. While conceptually sound, experimental validation of these differences remains lacking and difficult to undertake.

POSSIBLE STRATEGIES TO MITIGATE THE SEX GAP

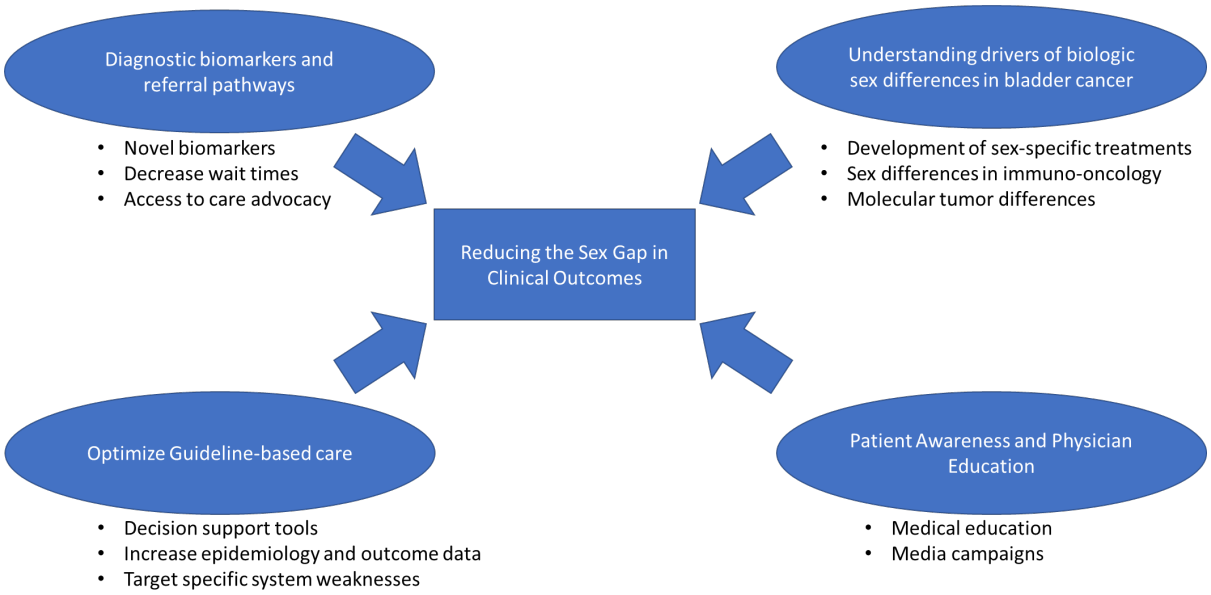
It is widely recognised that, despite little difference in treatment patterns or quality measures, female sex is associated with worse overall survival among individuals with MIBC[19]. However, although the UK data illustrated above highlight a sex gap in outcomes that is most apparent for stages 2 and above (≥pT2), such differences are not uniformly reported internationally. Differences in healthcare system access, cancer registry reporting, and treatment availability may explain the disparity between regions; publication bias in the available literature may also contribute to some discrepancies[105]. Given the differences in immunobiology outlined above, it is perhaps surprising that the reported sex differences in outcomes across both NMIBC and MIBC patients remain inconsistent and ambiguous, in contrast to the unequivocal dichotomy in incidence. See **Table 2**. Future initiatives should focus on root causes for sex-specific differences in pathological staging and features at diagnosis[46], as well as prospectively collecting relevant data[18] – the avoidance of stage migration subsequent to symptom ignorance or delayed referral to secondary care is fundamental[106].

Table 2: Bladder cancer associated phenomena that demonstrate confirmed differences between the sexes.

	Male	Female
Incidence[1] *Age standardized rate (ASR) per 100,000 persons per year*	9.6	2.4
Referral[21,29-34]	Prompt referral commonplace.	Delayed referral frequent.
Treatment	No differing recommendations between sexes. Differential responses potentially treatment- and territory-specific.	
Sex hormones[59,60,64-67,69-74]	Androgens may promote carcinogenesis via AR.	Differential roles of ERα and ERβ, equivocal role of estrogens.
Genomics[68,85,86]	Maintained *KDM6A* expression may be protective (most relevant in females). Methylation differences predominate.	
Histopathology & molecular subtypes[46,87,88]	For MIBC, urothelial cancer predominates, with higher proportion of luminal papillary and neuro-endocrine-like subtypes.	For MIBC, increased frequency of basal subtype and squamous carcinoma.
Immunology & microenvironment[91,95]	Evidence of AR-driven T cell exhaustion.	In NMIBC, immune exhaustion may result from increased immunosuppressive macrophage & B cell populations.

In recent years, increasing adoption of guideline-recommended treatments (e.g. neoadjuvant chemotherapy, trimodality therapy, etc.) may be attenuating such outcome differences between sexes. Notwithstanding, there is a general acceptance across the BC research field that much more work needs to be done to better understand the differences in disease incidence between males and females, and the seemingly worse outcomes for female MIBC patients[19,28]. See **Figure 4**.

Figure 4: An overview of different approaches to decrease the sex gap in bladder cancer outcomes. *Each domain and sub-domain likely require adequately-powered prospective studies in order to validate and implement strategies that can reduce the sex gap in clinical outcomes.*



Awareness of diagnostic differences between sexes

To avoid diagnostic and referral delays, clinicians need to be aware of the differential presentations of BC between sexes. Similar situations are present in other diseases. For example, symptoms experienced during acute coronary syndrome (ACS) differ between the sexes, with a higher proportion of women in those patients presenting without typical chest pain[107]. This leads to improved detection in men and worse comparative survival in women presenting with ACS; specific awareness drives have been funded in the UK to reduce this difference. For BC, raising awareness of haematuria as a sign of bladder cancer are also important. Previous work on symptom awareness (such as haematuria[108]) has suggested that mass media campaigns combined with targeted higher-intensity community-based programmes for high-risk populations (low socio-economic status, older age, and specific racial groups) can effect change[109]. Campaigns specifically aimed at women may be required.

Overall, the importance of haematuria in both sexes should be underlined in primary care where most patients initially present. Primary care physician education drives through presentations at conferences, decision support tools, and practice-specific presentations have been shown to improve referral rates for dementia[110] and their use for early diagnosis of BC should be investigated.

Addressing clinical & healthcare system factors

Addressing health system, access and referral issues leading to stage migration and sex differences in outcomes remains important. Compared to biological differences, problems such as delay in diagnosis and suboptimal treatment are readily modifiable factors to improve outcomes. However, it is important to understand the severity of these problems may vary by jurisdiction. For example, in an American National Cancer Database study, while women with MIBC had poorer overall survival, this difference did not appear to be related to measures of treatment quality, with data suggesting lower rates of treatment delay in women[46]. Notwithstanding, the BC diagnostic and treatment pathway is prolonged for all patients and strategies to reduce delays are urgently needed for all patients[111,112]. Accurate diagnostic urinary biomarkers[113,114] may facilitate timely urologic evaluation, and deployment in primary care may be particularly useful for the initial assessment of female haematuria patients.

Evidently, the relative contribution of delays in diagnosis and evaluation to differences in clinical outcomes is complex and difficult to dissect[115,116]. Although stratified by stage, the data in **Figure 1b** do not take account of the difference in outcomes between stages T1a and T1b, T2a and T2b, and T3a and T3b, to which these factors will contribute. Similarly, the extent to which significantly greater sex differences in stage are related to biological differences, access to healthcare, or delays in diagnosis cannot easily be determined with the available data. Socio-economic factors impacting access to care may drive some of the differences[116]. For example, it is striking that differences in the rate of MIBC at diagnosis between sexes according to SEER data is greater among African-Americans (30% males vs 43% females) versus Caucasians (22% vs 25%). In addition to potential biological drivers of these race differences, access to care is likely a major contributor based on similar data for other cancers[117]. Social barriers which limit access to care and timely referral are thus important to address. BC disproportionately affects patients with lower socio-economic status and research from Canada suggests that this gap has broadened, particularly among women[118]. Similar research elsewhere can help to highlight regional and national deficiencies which can stimulate policy and funding changes at a larger level.

As part of healthcare delivery, decision support tools (DSTs) may be useful due to their ability to be embedded within practice electronic medical systems so that they are easily accessible during consultation[119]. They can be automated to draw in background information on smoking status or family history to prompt clinicians to refer for investigation of a certain condition. In an evaluation of a 7-point checklist DST for the assessment of pigmented skin lesions, primary care physicians found such tools easy to use and particularly useful for borderline decision-making[120]. However, widespread use of DSTs relies on the levels of trust placed in the tools, compatibility of the DSTs with specific

electronic care systems, and difficulty in usage[119]. Furthermore, the ability of DSTs to affect change in cancer survival is uncertain and requires ongoing investigation. In the context of haematuria, models have been identified that could be used in primary care to guide referrals, with the potential to identify lower-risk patients with visible haematuria and to stratify individuals who present with non-visible haematuria[121]. A recent systematic review identified 13 such models with good discrimination for the diagnosis of bladder or kidney cancer (area under the receiver operating curve, AUROC >0.8), although only 8 had been externally validated; all of the studies had either high or unclear risk of bias[121]. The authors concluded that external validations in appropriate populations were required before implementation in primary care[121].

Optimising guideline-based care

The establishment and popularisation of guidelines for the referral of women with haematuria are vital to promote standards of care for referral to cystoscopy. Accounting for different age-adjusted cancer risks, some referral guidelines for haematuria differ between men and women[122]. For example, the American Urological Association guidelines give greater weight to non-visible haematuria in men versus women during the 5th and 6th decade of life[123]. However, the relative paucity of research data to establish these recommendations and the variability of current recommendations between jurisdictions contributes to potential confusion and uncertainty among primary care providers[30].

Should the standard of care for female patients with urothelial carcinoma treatment differ from male patients? This remains a challenging question, particularly as treatment patterns and practice evolves over time. Some historical data suggest that response to treatment may be inferior for women[11,15,52]. This includes a meta-analysis suggesting that female sex is associated with poorer cancer-specific survival and inferior responses to BCG. For NMIBC, when evaluated critically, we believe the most reliable and recent data do not support the notion that treatment guidelines should differ between men and women. In metastatic urothelial cancer, men and women appear to have similar treatment outcomes across various studies[124]. Therefore, despite the poorer outcomes reported for female patients with advanced BC, it is not clear that alternate standards of care are warranted.

Overall, accurate and reliable data are essential to identify the weak points in patients' journeys for which improvements in care can translate into better outcomes. This can be applied at both the health system and the hospital level. Equality (and diversity) in recruitment to clinical trials is also essential, with appropriate instigation of meta-analyses where data gaps exist.

Harnessing biological differences to improve treatment

A meaningful assessment of the fundamental sex differences in urothelial transformation and subsequent cancer biology is challenging as it transects the complex interplay of sex hormones, environmental exposures, microenvironment, immunology, genetics and genomics, and the microbiome. Nonetheless, this research is critical to identify specific differences which can be translated to clinical care – a call to action which has recently been emphasised[19]. However, in an era where molecular classification, personalised medicines and targeted therapies are endlessly sought, this 'simple' knowledge may remain years away – with approximately 1 in 4 patients female, many studies are underpowered to answer the question of whether a certain biomarker or classifier or treatment is effective or not in female patients.

To address some of these challenges, it is important to design studies accordingly[19]. For example, in pre-clinical studies, attention is needed to account for the sex of origin of cell lines and to include studies in both male and female mice. In clinical trials, reporting of results should include sex-based analyses. Furthermore, evaluating prognostic and predictive biomarkers for prognosis and response to therapy according to sex is necessary in correlative analyses of clinical trials. Notably, the recruitment of women into clinical trials is an important pre-requisite for these analyses as this has been a historical challenge. For instance, in a series of well-known NMIBC trials in Spain, only 11% of all recruited patients were women[125].

Biological differences may present unique opportunities to tailor treatment according to sex. Preclinical studies suggest that alterations in sex steroids with AR antagonism may represent a strategy to treat male bladder tumours alone or in combination with immunotherapy. Based on initial pre-clinical studies, AR antagonism may alter immune responsiveness to immunotherapy through alterations in the steroid milieu[92]. Hormonal differences are broadly recognized to impact the immune response[126], and AR suppressive therapy may improve BC outcomes through a hormonal-mediated modulation of the TME[127]. Furthermore, there is now a significant amount of clinical data to suggest that 5-alpha reductase inhibitors (5ARIs) could decrease the incidence and recurrence rates of low-grade BC in men. With a 2021 meta-analysis suggesting a HR of 0.46 for recurrence for male NMIBC patients receiving 5ARI therapy, prospective clinical trials are warranted. However, these should be adequately powered to avoid false-negatives from short-term evaluation of a long-acting mechanism of action[128].

Future directions

Research and advocacy are important to address the sex gaps in clinical care. Improved data collection on the natural history and epidemiologic difference in haematuria between sexes can drive the development of best practices and referral pathways[18]. Similarly, understanding the gaps in referral can facilitate targeted campaigns to raise awareness in both the populations at risk and their healthcare providers.

At a more fundamental level, there exist many opportunities to expand our understanding of how sex differences impact the TME of BC and the immune interactions which contribute to anti-cancer activity and response to immune-targeted treatments. Understanding immune differences at both the level of the urothelial and systemic interactions may facilitate more effective and personalised therapies.

Clinical research is now emerging to evaluate whether sex-specific treatments are effective, such as suppression of the androgen axis in combination with existing treatments (e.g. the 'BicaBCa' study, NCT05327647) in men. Further studies to target sex-specific strategies to decrease recurrences of NMIBC or progression of MIBC are similarly warranted given existing data[127], and increased reporting of sex-based analyses in clinical trials is to be welcomed. Importantly, improved awareness of the sex gap is also apparent, as illustrated by a number of presentations at this year's American Society of Clinical Oncology Genitourinary cancers symposium[129-131].

CONCLUSIONS

In conclusion, addressing the sex gap in BC outcomes requires coordinated efforts to improve outcomes for women. A greater understanding of the sexual dimorphism of BC biology and immunology may permit personalised, sex-specific biomarkers. There is also a need to develop sex-specific treatments through clinical trials, such as treatments targeting the androgen axis. In parallel, it is important to recognise that implementation of best practices for referral, diagnosis and treatment of BC can provide rapid improvements in outcomes where deficiencies exist. Further research is also needed to identify optimal strategies for the referral and evaluation of haematuria between sexes.

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COMPETING INTERESTS

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KEY POINTS

- Bladder cancer is a common malignancy with a 3-4 to 1 male preponderance, suggesting relative 'resistance' to the development of the disease in females compared to males. Environmental exposures, hormone signalling, gene expression, immunology and tumour microenvironment likely coalesce to underpin this sexual dimorphism.
- Although bladder cancer outcomes are considered to be notably worse for females than males, this finding is not consistent across territories and treatment modalities, suggesting the considerable influence of healthcare system factors on outcomes. Such factors may include diagnostic delays and discrepancies in the appropriate and timely use of guideline-based care.
- Addressing healthcare system factors by the implementation of best practices for referral, diagnosis and treatment could provide rapid improvements in outcomes where deficiencies exist.
- Nevertheless, the biological phenomena driving the sexual dimorphism in disease incidence are likely to also influence treatment responses, and better understanding of these mechanisms through carefully-designed fundamental research, and preclinical and clinical studies, may reveal sex-specific biomarkers or treatment approaches to benefit all bladder cancer patients.

TABLE & FIGURE LEGENDS

Table 1: Summary of the main characteristics of the consensus classes of muscle invasive bladder cancer (MIBC) (adapted from[77]).

Table 2: Bladder cancer associated phenomena that demonstrate confirmed differences between the sexes.

Figure 1: 1-year, 2-year, 3-year, and 4-year bladder cancer survival (%) for adults (aged 15 to 99 years) diagnosed in England 2015 to 2019, followed up to 2020[17]. **(a)** Age-standardised and non-age standardised net survival (%) by sex. **(b)** Age-standardised net survival (%) by stage and sex. **NB:** 3-Year Stage 1, 3-Year Stage 4, 4-Year Stage 1, 4-Year Stage 3 and 4-Year Stage 4 data are all missing for female patients. Age-standardisation represents a weighted-average of mortality rates for each sex based on the International Classification of Survival Standard.

Figure 2: Overall survival for patients with T1–T4 bladder cancers treated in England from 2013 to 2019, plotted using the Kaplan-Meier method with respect to sex (reproduced with kind permission from John Wiley and Sons[18]).

Figure 3: A summary of immunological sex differences in BC: The female TM) is characterised by increased infiltration of immunosuppressive CD163+ M2-like macrophages and increased tumour expression of immune checkpoints. In addition, an increase in genes related to B cell recruitment (CXCL13) and function (CD40) has been shown in the female TME. In contrast, the male TME is characterised by increased exhausted CD8+ T cells (both of progenitor and terminally differentiated subtypes), which is driven by AR signalling specifically in CD8+ T cells. **AR**: androgen receptor, **IFN**: interferon, **TME**: tumour microenvironment.

Figure 4: An overview of different approaches to decrease the sex gap in bladder cancer outcomes. Each domain and sub-domain likely require adequately-powered prospective studies in order to validate and implement strategies that can reduce the sex gap in clinical outcomes.

SHORT SUMMARY

Bladder cancer outcomes are considered worse for women than for men, while incidence is 3-4 times higher in men. Understanding biological phenomena and health system factors driving these differences is essential to improve outcomes and develop novel treatment approaches.