

The sex gap in bladder cancer survival

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DOI:

[10.1038/s41585-023-00806-2](https://doi.org/10.1038/s41585-023-00806-2)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Toren, P, Wilkins, A, Patel, K, Burley, A, Gris, T, Kockelbergh, R, Lodhi, T, Choudhury, A & Bryan, RT 2023, 'The sex gap in bladder cancer survival: a missing link in bladder cancer care?', *Nature reviews. Urology*. <https://doi.org/10.1038/s41585-023-00806-2>

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

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34

35 **ABSTRACT**

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

55

56

57 INTRODUCTION

58 Bladder cancer (BC) is the tenth most common cancer worldwide, responsible for 3% of annual cancer
59 diagnoses and 2.1% of cancer-related deaths¹. Most patients (75-80%) present with non-muscle-
60 invasive bladder cancer (NMIBC: stages Ta/T1/Tis) – up to 80% of these patients will experience
61 recurrence², and up to 44% will progress to muscle-invasive bladder cancer (MIBC: stages T2-4)²⁻⁴.
62 Moreover, of the 20-25% of patients initially diagnosed with MIBC, around one-quarter will have
63 incurable, locally-advanced or metastatic disease⁵. Muscle-invasion thus represents a critical step in
64 the disease course, carrying a 5-year survival of only 27-50%, despite radical therapies³.

65 Overall, the number of incident BC cases continues to increase, although the age-standardized
66 incidence and mortality appear to be decreasing, paralleled by fewer smoking-related cases⁶. Smoking
67 is the largest modifiable risk factor for BC, with exposure to occupational carcinogens also well
68 documented⁷. However, sex is the largest risk factor for BC, with men 3-4 times more likely to be
69 diagnosed with BC than women^{8,9}. Furthermore, there is no clear indication of the differential effects
70 of smoking between men and women¹⁰. Nonetheless, women tend to have more aggressive tumours
71 at diagnosis and experience worse outcomes thereafter¹¹⁻¹⁵.

72 BC cases which are muscle-invasive or metastatic at presentation contribute the most significant
73 morbidity and mortality, while the surveillance of NMIBC drives BC to be one of the most expensive
74 per patient cancers to treat¹⁶. Therefore, there is a need to identify and fill the sex-based gaps in our
75 understanding and care for all BC patients. In this perspective, we review the differences in outcomes
76 between men and women with BC, discuss our current understanding of these differences, and
77 propose solutions to pursue. These differences are significant given the impact of BC on patients and
78 healthcare systems.

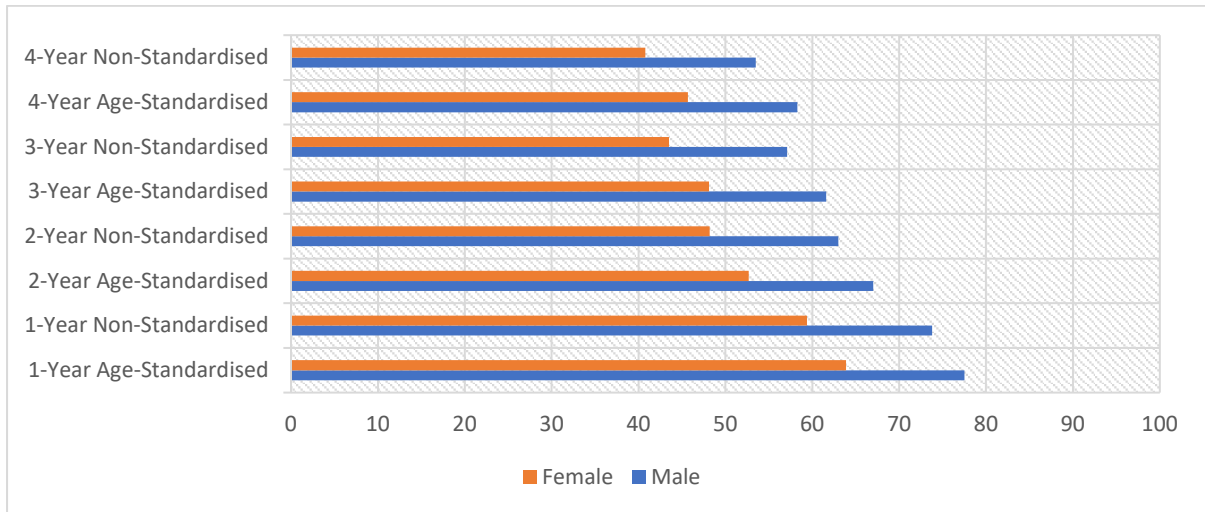
79

80 SEX DIFFERENCES IN OUTCOMES

81 Recent data regarding cancer survival in England (cancers diagnosed from 2015 to 2019, followed up
82 to 2020¹⁷) appear to lay bare the persistent sex gap in survival for BC patients. Across all stages of BC
83 and for 1-, 2-, 3- and 4-year BC survival, female patients had worse outcomes than male patients
84 (**Figure 1**). With some 3- and 4-year stage-specific survival data missing for female patients (only), a
85 knowledge deficit may already be apparent. Notwithstanding, using an overlapping dataset available
86 for T1–T4 tumours treated from 2013 to 2019, recent analyses of overall survival for urothelial cancer
87 patients in England by Catto et al also illustrate a similar situation¹⁸. Beyond the UK, such gaps in
88 outcomes continue to be highlighted internationally¹⁹⁻²³.

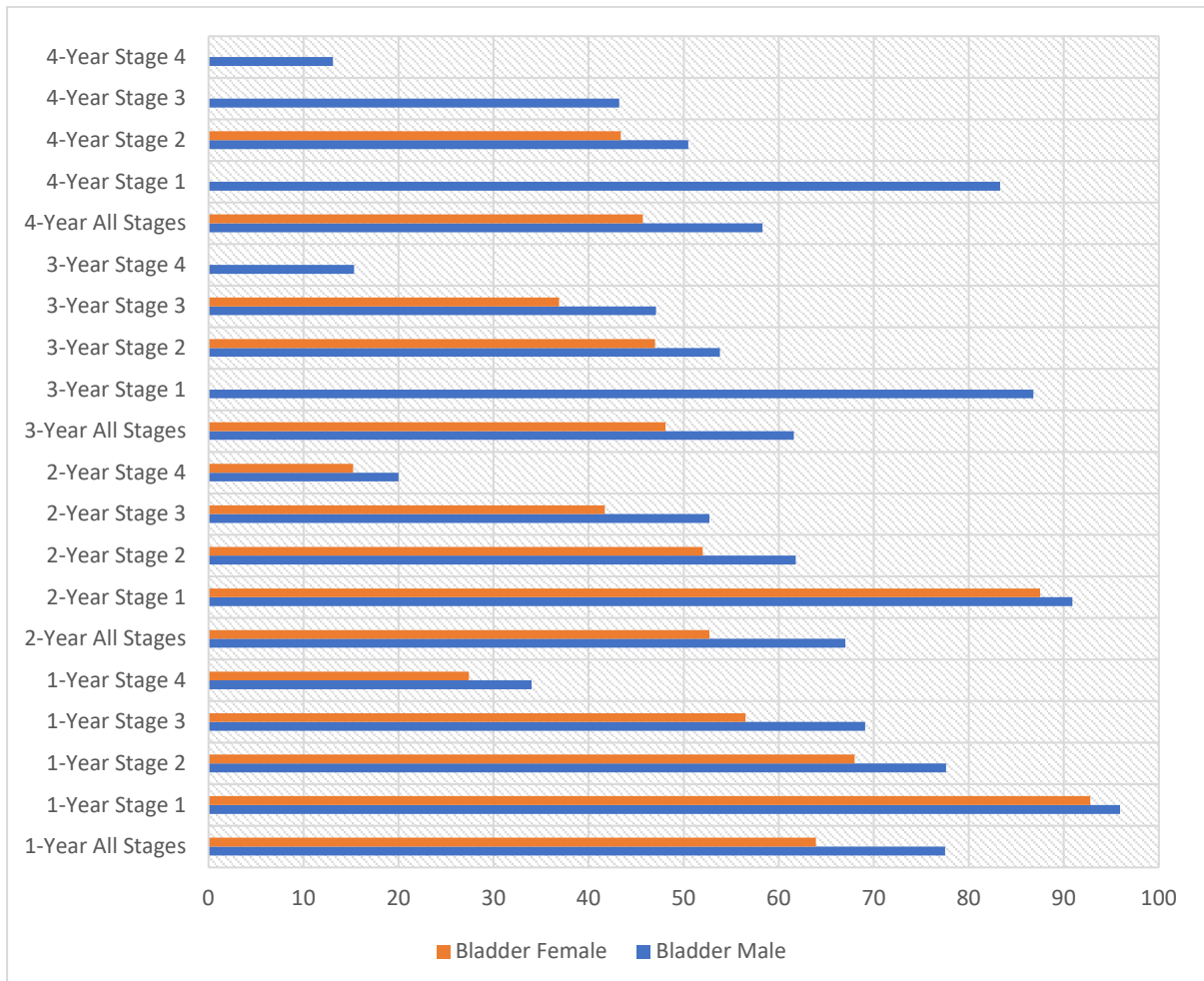
89 **Figure 1:** 1-year, 2-year, 3-year, and 4-year bladder cancer survival (%) for adults (aged 15 to 99 years)
 90 diagnosed in England 2015 to 2019, followed up to 2020¹⁷. **(a)** Age-standardised and non-age
 91 standardised net survival (%) by sex. **(b)** Age-standardised net survival (%) by stage and sex. **NB:** 3-
 92 Year Stage 1, 3-Year Stage 4, 4-Year Stage 1, 4-Year Stage 3 and 4-Year Stage 4 data are all missing
 93 for female patients. Age-standardisation represents a weighted-average of mortality rates for each
 94 sex based on the International Classification of Survival Standard.

95 **(a)**



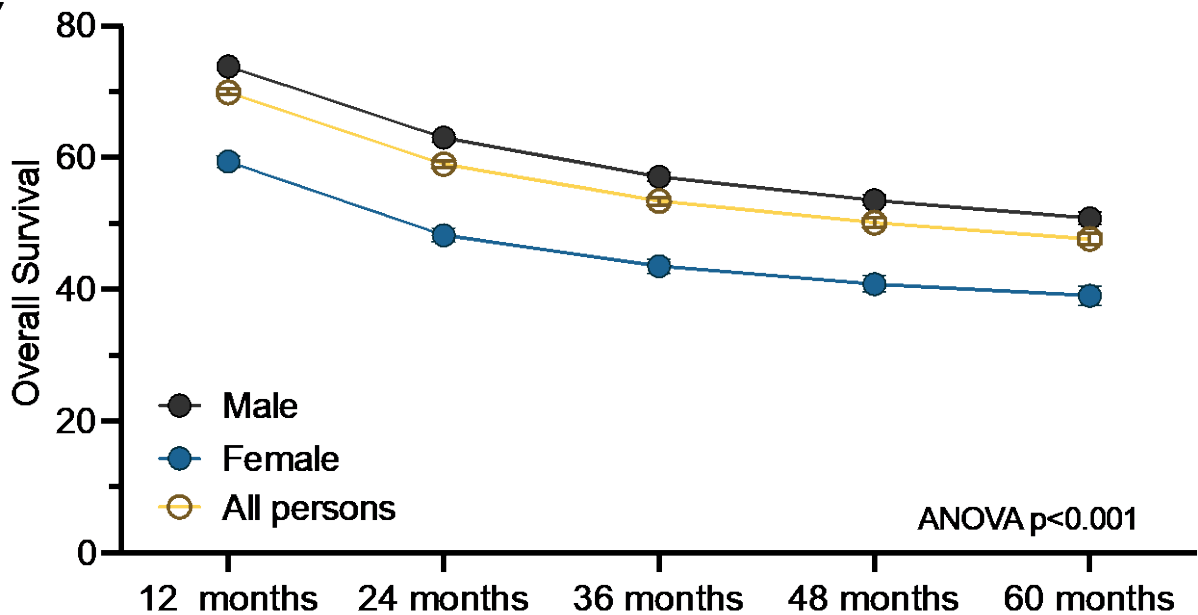
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97 **(b)**



98

99 **Figure 2:** Overall survival for patients with T1–T4 bladder cancers treated in England from 2013 to
 100 2019, plotted using the Kaplan-Meier method with respect to sex (reproduced with kind permission
 101 from John Wiley and Sons¹⁸).



102

103

104 This is neither a new phenomenon, nor an unexplored phenomenon^{19,22}, yet it remains unexplained
 105 worldwide. Moreover, these findings in BC are the inverse of the situation for most cancer sites where
 106 cancer outcomes are commonly worse for men²⁴. Previous European data from 1999-2007 also
 107 reported lower BC survival among women, driven by differences in Northern and Central European
 108 countries and the UK²⁵. More recent data from the Netherlands also mirrors the UK data²⁶. However,
 109 other studies have found no differences in treatment or cancer-specific survival²⁷. A 2022 systematic
 110 review and meta-analysis concluded that female sex was associated with worse cancer-specific
 111 survival and overall survival in patients with MIBC, but no differences were apparent in the relatively
 112 lower number of studies with sex-specific outcomes for NMIBC²⁸. Concordant with the recent NHS
 113 results, this suggests that sex differences in survival are principally driven by patients with non-
 114 metastatic MIBC at presentation, or those who progress to develop MIBC.

115

116 POSSIBLE REASONS FOR DIFFERENCES IN OUTCOMES

117 The possible explanations are complex – the interplay of the signs and symptoms of the disease, speed
 118 of referral into the BC diagnostic pathway, treatment decisions, treatment responses, and biology²⁹.
 119 It is important to better understand the weight and relevance of each of these factors in order to take
 120 concrete steps to further investigate and potentially close the apparent gap in overall outcomes.

121 **Recognising the importance of haematuria**

122 Female patients may not recognise the importance of haematuria in a disease where there is a 3:1
123 male preponderance or may not seek investigation as early²¹. Haematuria is the most common
124 symptom of bladder cancer². However, from a young age, women are much more likely to present
125 with a UTI and have associated haematuria in some instances. This can desensitize both patients and
126 clinicians to not recognize the importance of haematuria, particularly in older women at higher risk of
127 bladder cancer³⁰.

128

129 **Referral for investigations**

130 Once haematuria has been identified as a potential symptom of bladder cancer, there is a need for
131 prompt referral according to guidelines. The preponderance of haematuria related to urinary tract
132 infection in women can result in delays in diagnosis – as reported internationally, female patients
133 presenting with haematuria to their primary care provider are not referred for subsequent
134 investigations as rapidly as male patients or are investigated less thoroughly^{21,29-34}. Indeed,
135 Lyratzopoulos *et al.* found that women had a significantly higher number of pre-referral consultations
136 than men when presenting with haematuria, with 27% of women requiring 3 or more consultations
137 compared with only 11% of men requiring the same³¹.

138

139 **Treatment decisions**

140 In the setting of NMIBC, sex does not appear to influence the utilisation of adjuvant intravesical
141 therapy³⁵, nor the choice of radical treatment for MIBC³⁶⁻³⁸ and, in terms of overall treatment
142 paradigms, there are currently no differing recommendations between sexes. Although the use of
143 continent urinary diversions appears to be lower in female patients than male patients^{39,40}, this does
144 not alter survival outcomes. This may reflect higher rates of advanced tumour stage in women, but
145 also potential differences in training and practice patterns, with fewer centres worldwide equally
146 comfortable to perform orthotopic continent diversion in women given anatomical and surgical
147 differences pertinent to differences in tumour stage. However, for female patients undergoing radical
148 cystectomy and bladder substitution, preservation of the uterus and attempted nerve-sparing appear
149 to result in better functional outcomes⁴¹, yet there remain significant gaps in the adoption of female
150 reproductive organ-sparing and nerve-sparing radical cystectomy techniques for patients with organ-
151 confined disease⁴². With emerging data from large series confirming the low rate of female
152 reproductive organ involvement at the time of radical cystectomy (4.2-5.7%)^{43,44}, there appear to be
153 potential sex disparities driven by provider expertise and preference. These disparities may need to
154 be addressed through training and/or the refinement or centralisation of specific provider expertise.

155 **Treatment efficacy**

156 Evaluating whether female patients derive less benefit from current treatments is more difficult to
157 dissect and may be, in part, driven by biological and anatomical reasons elucidated in the section
158 below. Female patients nonetheless present with worse disease stage^{9,33,38,45}, and more often with
159 non-urothelial tumour histology^{9,20,46}, thus contextualising subsequent sex-specific treatment
160 responses. Studying 24,169 BC patients in The Netherlands, Richters *et al.* found that, in the first two
161 years after diagnosis, excess mortality rates for women were higher than for men but lower
162 thereafter⁹. This applied to both NMIBC and MIBC patients, and baseline differences in age, stage, and
163 histology accounted for only part of the excess mortality gap⁹. Ballas *et al.* reported similar findings
164 for patients undergoing bladder preservation (trimodality therapy) for T2-T4a N0 M0 MIBC⁴⁷.
165 Marinaro *et al.* studied 47,229 MIBC patients in the USA and identified increased 90-day mortality
166 following radical cystectomy and worse overall survival in female patients³⁷. Others have also
167 highlighted either worse cancer-specific survival in females than males following radical
168 cystectomy^{36,45}, or both worse cancer specific and overall survival^{11,48}. Notwithstanding, other
169 research suggests that higher uptake of neoadjuvant chemotherapy diminishes these sex
170 differences⁴⁹. Such data may indicate that if sex differences in outcomes are attenuated in patients fit
171 enough for chemotherapy then, simply by selection bias, similar outcomes could also be expected in
172 the generally fitter patients enrolled into clinical trials. In the radiotherapy setting, unpublished data
173 from Manchester, UK, regarding 209 MIBC patients treated by radiotherapy with concurrent carbogen
174 and nicotinamide (BCON protocol⁵⁰) show no difference in 5-year cancer-specific survival between
175 males and females.

176 In addition to overall survival data, conflicting data report on potential sex differences in response to
177 specific treatments for BC⁵¹. Evaluating response to intravesical treatment for NMIBC, earlier reports
178 (summarised in a 2018 meta-analysis and systematic review⁵²) suggest women have poorer responses
179 to BCG. However, there appears to be publication bias in this meta-analysis toward studies reporting
180 a sex-based difference and, thus, there is doubt as to these conclusions suggesting differential
181 responses in NMIBC to BCG⁵². In line with this, two large contemporary cohorts of BCG-treated NMIBC
182 patients did not report any sex differences in outcomes of recurrence-free survival or progression-free
183 survival^{53,54}. Furthermore, the literature suggests no sex differences in response to intravesical
184 chemotherapy for NMIBC.

185 For advanced BC, the accumulating evidence does not suggest that sex-based differences exist in
186 disease response to immune checkpoint blockade (ICB); this includes the sex-based analyses
187 presented in recent trials, including KEYNOTE-052⁵⁵, KEYNOTE-361⁵⁶, and IMvigor130⁵⁷. Moreover, the
188 best available data do not suggest sex-specific differences in response to chemotherapy, with Haines

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

195

196 **Biological and anatomical phenomena**

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

203 Sex hormones

204 Knockout studies in mice suggest that the androgen receptor (AR) in the urothelium is important for
205 urothelial carcinogenesis^{59,60}. Further, estrogen receptor alpha (ER α) and estrogen receptor beta (ER β)
206 knockout mice experiments suggest that ER α has a protective role against BC initiation and
207 progression, while ER β could promote BC⁶¹⁻⁶³. These murine results are consistent with
208 immunohistochemical studies demonstrating downregulated ER α expression in higher grade and
209 higher stages bladder tumors⁶⁴ and upregulated ER β and aromatase protein expression in higher grade
210 and more aggressive bladder tumors⁶⁴⁻⁶⁷. Using the “four cores genotypes” mouse model, which
211 decouples chromosomal and gonadal sex, researchers found gonadal sex (i.e. hormonal exposure) had
212 the single largest impact on chemically-induced bladder tumour development⁶⁸; notwithstanding,
213 chromosomal sex also independently influenced tumour development⁶⁸.

214 Sex steroids have direct effects on the activity and function of various subsets of innate and adaptive
215 immune cells and are known to contribute to immunological differences between sexes. The role of
216 systemic hormones in the incidence and progression of bladder tumours remains incompletely
217 defined, particularly in women. Epidemiologic studies of BC among women suggest a later age at
218 menopause, parity (vs nulliparity) and use of hormone replacement therapy (HRT) may be associated
219 with decreased BC incidence⁶⁹⁻⁷³. However, a meta-analysis suggested that the age of menarche does
220 not affect the risk of BC in women⁷⁴, and so a need for clarification remains.

221

222 Genomics

223 The genomic and molecular understanding of BC has advanced in recent years⁷⁵⁻⁷⁹. MIBCs are
224 heterogeneous⁸⁰ and characterised by many single nucleotide variants (SNVs) and copy number
225 variants (CNVs)^{75,81,82}; loss of multiple tumour suppressors and alteration of multiple pathways are
226 common^{77,78}. Six consensus gene expression-based subtypes of MIBC are now recognised and share
227 some characteristics⁷⁷, but which remain heterogeneous with respect to genomic aberrations and
228 behaviour; temporal and spatial plasticity in subtype has also been reported⁸³. See **Table 1** below.
229 NMIBC is arguably more complex than MIBC^{78,79}, comprising multiple grades of disease⁸⁴.

230 Within this landscape, BCs (alongside other cancers) demonstrate extensive sex-biased molecular
231 signatures, with sex-biased expressed genes enriched in the sex chromosomes and evidence of sex-
232 biased DNA methylation patterns (e.g. *TOP2B*)⁸⁵. Sex-biased pathways include those related to
233 immune responses, apoptosis and the cell cycle, metabolism, DNA repair and P53 pathways⁸⁵.
234 Furthermore, *KDM6A* alterations are common in BCs (24-33%^{75,79}) with the gene functioning as an
235 epigenetic regulator of downstream gene expression; importantly, *KDM6A* escapes X chromosome
236 inactivation¹⁹. Mouse models appear to demonstrate that loss of *Kdm6a* increases BC risk in female
237 mice, and mutations or reduced expression of human *KDM6A* predicts poor prognosis in female BC
238 patients⁶⁸. Other research has suggested a higher rate of *KDM6A* mutations in NMIBCs from female
239 patients⁸⁶.

240

241 **Table 1: Summary of the main characteristics of the consensus classes of MIBC (adapted from⁷⁷).**

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

242

243

244 Histopathology & molecular pathology

245 In BC pathology, there are several notable differences between sexes. A review of over 27,000 patients
 246 in the National Cancer Database in the US found women have more non-urothelial carcinoma (15.1%
 247 vs 9.9%), with squamous carcinoma the predominant histology⁴⁶. Similarly, UK national data from over
 248 100,000 T1-T4 BC patients demonstrated that women had more non-urothelial cancer than men (27%
 249 vs 16%, respectively)⁸⁷. For MIBC, women appear to have a higher proportion of tumours with a basal
 250 molecular subtype, while men have a higher proportion of luminal papillary and neuro-endocrine-like
 251 subtypes⁸⁸. However, an analysis of NMIBC did not identify differences in molecular subtypes
 252 according to sex⁸⁹.

253

254

255 Tumour immunology & microenvironment

256 The wide repertoire of immunomodulatory agents used across stages for treatment of BC, including
257 in clinical trials, means that the contribution of sex-specific immunological and microenvironmental
258 factors is likely to be clinically important. Such factors can include genetic, epigenetic and
259 transcriptional effects⁸⁵, which may relate to XX and XY chromosomal differences⁹⁰, as well as
260 hormonal effects⁹¹. Several pre-clinical and clinical studies highlight these factors and provide some
261 explanation for the divergent observations of increased incidence of BC in male populations yet
262 inferior survival outcomes in women described earlier.

263 Studies of bladder tumour AR expression do not show sex-based differences in expression⁹². However,
264 Kwon *et al.* have recently described a fascinating androgen-driven mechanism of T cell exhaustion in
265 BC⁹¹. Their insights provide some explanation for why spontaneous rejection of early immunogenic
266 bladder tumours is less common in males, and hence the male predisposition for the development of
267 BC. The study evaluates three different bladder tumour models, MB49 (transplantable syngeneic
268 tumours), BBN (carcinogen-induced tumours) and BKL171 where BBN-induced tumours develop in a
269 testis-bearing mouse with an XX chromosome to eliminate any immune response to male-specific
270 minor antigens. The authors demonstrate more aggressive tumour growth in male versus female mice
271 with these three models⁹¹. This effect was eliminated using Tcrb/Tcrd or RAG2 knockout mice, which
272 specifically lack T cells, and reinstated with adoptive transfer of CD8⁺ T cells. A two-fold higher
273 frequency of polyfunctional CD8⁺ T cells able to produce Interferon gamma (IFN γ), Tumour Necrosis
274 Factor Alpha (TNF α) and Granzyme B (Gzmb) was seen in MB49 tumours of female versus male mice
275 at day 9 of tumour growth.

276 In support of the above observations, single-cell RNA sequencing identified increased effector-like
277 CD8⁺ T cells in the female versus male tumour microenvironment (TME). In contrast, the male TME
278 was enriched for progenitor exhausted CD8⁺ tumour infiltrating lymphocytes (TILs), as defined by their
279 stem-like genetic profile (i.e. Tcf1/Tcf7⁺). These TILs showed accelerated progression to terminally
280 differentiated Tcf1-Tim3⁺ exhausted T cells incapable of restimulation. In keeping with all of the above
281 results, male mice with loss of AR exclusively in CD8⁺ T cells were equally protected against cancer as
282 female mice. Finally, the authors observe a negative correlation between type I interferon signalling
283 and AR activity in T cells and they suggest this balance may underlie wider sexual dimorphism in cancer
284 immunity. Insights from the above study are corroborated by observations elsewhere that androgen
285 deprivation therapy can promote responses to ICB^{92,93}. The work by Kwon *et al.* is CD8⁺ T cell specific;
286 CD4⁺ T cells are known to be important in BC immunology⁹⁴, and there are likely further sex-specific
287 mechanistic insights to T cell biology to be uncovered in the future.

288 Studies to explore a biologic basis for different responses to BC therapy have yielded interesting
289 results. An analysis of the whole transcriptomes of 460 tumours from the UROMOL cohort, together
290 with multiplex immunofluorescence of tumours from the Kingston Health Services Centre (KHSC)
291 cohort (n=332, 22% female)⁹⁵ reported increased expression of the immune checkpoint genes *CTLA4*,
292 *PDCD1*, *LAG3*, and *ICOS* in high-grade tumours from females compared to high-grade tumours from
293 males or low-grade tumours from either sex. In addition, increased expression of CXC ligand 13
294 (*CXCL13*, an important B-cell–recruiting chemokine) and the B-cell surface-associated molecule *CD40*
295 were seen more frequently in high-grade tumours from female patients. Intriguingly, the authors also
296 observed increased infiltration of CD163⁺ M2-like tumour-associated macrophages (TAMs) in both
297 low- and high-grade NMIBC tumours of female patients versus those of male patients⁹⁵. Of further
298 clinical relevance, a higher density of CD163⁺ M2-like TAMs and CD79a⁺ B cells was independently
299 associated with shorter recurrence-free survival across all high-grade tumours in the KHSC cohort
300 supporting a functional relevance of this sexually dimorphic observation. Together, these findings
301 suggest that the TME of NMIBC from female patients tends towards greater immune exhaustion
302 where immune dysfunction is accentuated by reciprocal communication between increased
303 immunosuppressive macrophage and B cell populations.

304 Elsewhere, research findings indicate that estrogen inhibits interleukin-6 and thus decreases the
305 expression of receptor complexes required for BCG adherence to urothelial cells, such as integrin
306 $\alpha 5\beta 1$ ^{96,97}. A therapeutic strategy combining the anti-estrogen therapy ICI 182780 with BCG was shown
307 to improve treatment efficacy in *in vitro* and *in vivo* pre-clinical systems, in part via enhancing TNF α
308 signalling⁹⁸. Furthermore, the immune checkpoint ICOS shows greater upregulation in whole blood
309 following stimulation of healthy female volunteers versus their male counterparts with BCG⁹⁹.
310 However, these findings require careful interpretation in view of the considerable immunological
311 differences between post-menopausal women and younger healthy volunteers. Overall, a current key
312 research gap is to understand the sex-specific longitudinal innate and adaptive immune changes that
313 occur both intra-tumourally and systemically over the months to years following BCG treatment,
314 including how these relate to a differential therapeutic response.

315 Immunological ageing or immunosenescence is known to have sex-specific biological characteristics¹⁰⁰.
316 A recent study using bulk RNA sequencing showed an enrichment for B cell function-associated
317 pathways in aged healthy female mice bladders versus their aged male counterparts¹⁰¹. Multiplex
318 immunofluorescence confirmed a greater number of organised tertiary lymphoid structures (TLS) in the
319 healthy bladders of female mice. Somewhat surprisingly, in murine bladders treated with several weeks
320 of the carcinogen BBN, there was no difference in TLS between male and female mice. Instead, an

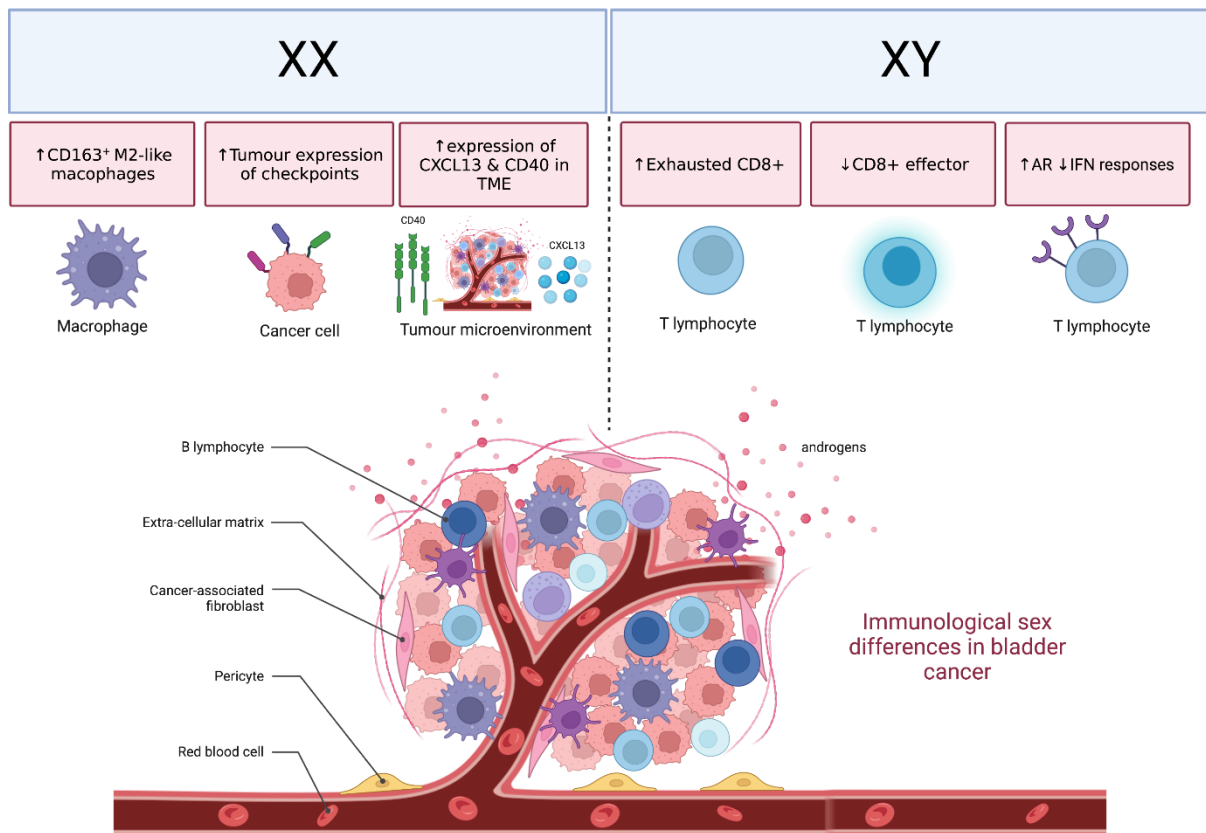
321 increase in plasma cells was seen in the lamina propria of female aged mice, and female mice had a more
 322 immune-infiltrated and oedematous lamina propria across ages.

323 As outlined earlier, a difference in the response to ICB according to sex has not been clearly established
 324 in BC. However, we know that immune cell PD-L1 is associated with inferior survival outcomes across
 325 sexes, and that androgen signalling in T cells represses IFN γ to limit ICB responses⁹³. Furthermore,
 326 estrogen plus a number of X-linked micro-RNAs, including miR-221, miR-222, and miR-106b, can
 327 regulate PD-L1 expression^{90,102}. Our incomplete understanding of the sex-specific immunogenomic
 328 changes underlying differential responses to ICB urgently warrants further research to optimise novel
 329 combination strategies across disease stages. See **Figure 3**.

330

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

338



339

340

341

342 Microbiome

343 Current research seeks to define whether differences in the urinary, tumoral or gut microbiome
344 between men and women may contribute to differential outcomes¹⁰³. While urinary microbiome
345 differences may be associated with the risk of recurrent urinary tract infections (UTIs) contributing to
346 carcinogenesis, the causal implication of potential microbiome differences in tumour progression or
347 treatment response remains to be defined²².

348 Anatomy

349 Anatomically, men have more outlet obstruction related to prostatic enlargement and subsequent
350 detrusor hypertrophy. Women have thinner bladder walls¹⁰⁴, which may help explain a higher
351 incidence of non-organ-confined disease at diagnosis⁴⁵. Differential urinary dwell times in men and
352 women may contribute to differences in BC development, with men more commonly having higher
353 post-void residuals with age. While conceptually sound, experimental validation of these differences
354 remains lacking and difficult to undertake.

355

356 **POSSIBLE STRATEGIES TO MITIGATE THE SEX GAP**

357 It is widely recognised that, despite little difference in treatment patterns or quality measures, female
358 sex is associated with worse overall survival among individuals with MIBC¹⁹. However, although the
359 UK data illustrated above highlight a sex gap in outcomes that is most apparent for stages 2 and above
360 (\geq pT2), such differences are not uniformly reported internationally. Differences in healthcare system
361 access, cancer registry reporting, and treatment availability may explain the disparity between
362 regions; publication bias in the available literature may also contribute to some discrepancies¹⁰⁵. Given
363 the differences in immunobiology outlined above, it is perhaps surprising that the reported sex
364 differences in outcomes across both NMIBC and MIBC patients remain inconsistent and ambiguous,
365 in contrast to the unequivocal dichotomy in incidence. See **Table 2**. Future initiatives should focus on
366 root causes for sex-specific differences in pathological staging and features at diagnosis⁴⁶, as well as
367 prospectively collecting relevant data¹⁸ – the avoidance of stage migration subsequent to symptom
368 ignorance or delayed referral to secondary care is fundamental¹⁰⁶.

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

	Male	Female
Incidence ¹ <i>Age standardized rate (ASR) per 100,000 persons per year</i>	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 ER α and ER β , equivocal role of estrogens.
Genomics ^{68,85,86}	Maintained <i>KDM6A</i> 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.

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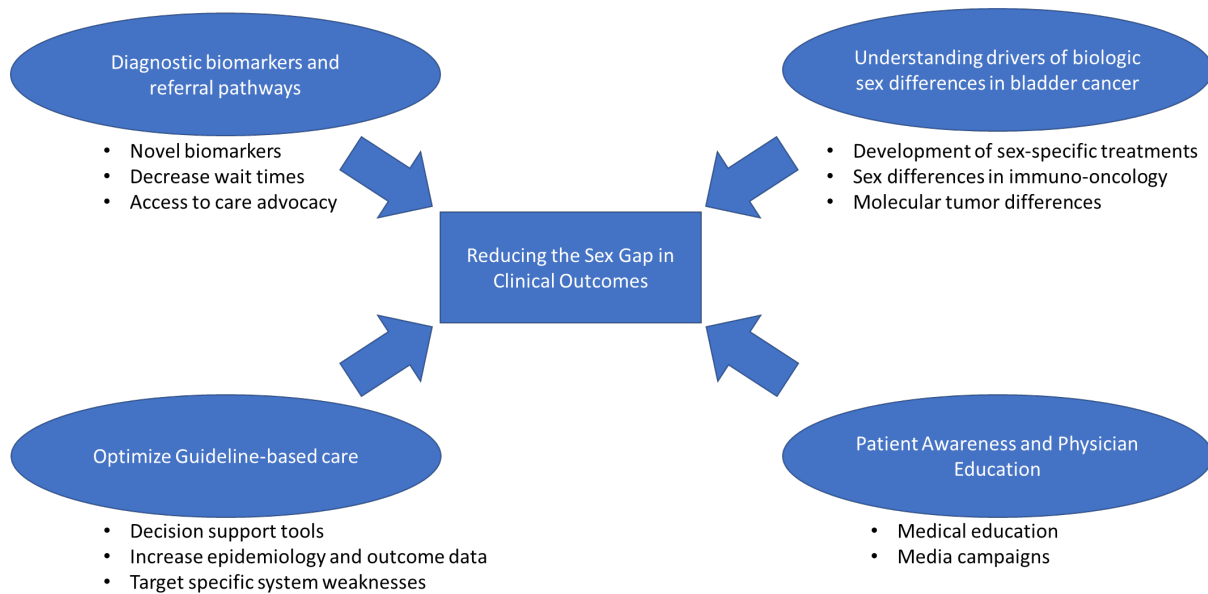
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375 In recent years, increasing adoption of guideline-recommended treatments (e.g. neoadjuvant
 376 chemotherapy, trimodality therapy, etc.) may be attenuating such outcome differences between
 377 sexes. Notwithstanding, there is a general acceptance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

380

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

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388 **Awareness of diagnostic differences between sexes**

389 To avoid diagnostic and referral delays, clinicians need to be aware of the differential presentations
 390 of BC between sexes. Similar situations are present in other diseases. For example, symptoms
 391 experienced during acute coronary syndrome (ACS) differ between the sexes, with a higher proportion
 392 of women in those patients presenting without typical chest pain¹⁰⁷. This leads to improved detection
 393 in men and worse comparative survival in women presenting with ACS; specific awareness drives have
 394 been funded in the UK to reduce this difference. For BC, raising awareness of haematuria as a sign of
 395 bladder cancer are also important. Previous work on symptom awareness (such as haematuria¹⁰⁸) has
 396 suggested that mass media campaigns combined with targeted higher-intensity community-based
 397 programmes for high-risk populations (low socio-economic status, older age, and specific racial
 398 groups) can effect change¹⁰⁹. Campaigns specifically aimed at women may be required.

399 Overall, the importance of haematuria in both sexes should be underlined in primary care where most
 400 patients initially present. Primary care physician education drives through presentations at
 401 conferences, decision support tools, and practice-specific presentations have been shown to improve
 402 referral rates for dementia¹¹⁰ and their use for early diagnosis of BC should be investigated.

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 404

405 **Addressing clinical & healthcare system factors**

406 Addressing health system, access and referral issues leading to stage migration and sex differences in
407 outcomes remains important. Compared to biological differences, problems such as delay in diagnosis
408 and suboptimal treatment are readily modifiable factors to improve outcomes. However, it is
409 important to understand the severity of these problems may vary by jurisdiction. For example, in an
410 American National Cancer Database study, while women with MIBC had poorer overall survival, this
411 difference did not appear to be related to measures of treatment quality, with data suggesting lower
412 rates of treatment delay in women⁴⁶. Notwithstanding, the BC diagnostic and treatment pathway is
413 prolonged for all patients and strategies to reduce delays are urgently needed for all patients^{111,112}.
414 Accurate diagnostic urinary biomarkers^{113,114} may facilitate timely urologic evaluation, and
415 deployment in primary care may be particularly useful for the initial assessment of female haematuria
416 patients.

417 Evidently, the relative contribution of delays in diagnosis and evaluation to differences in clinical
418 outcomes is complex and difficult to dissect^{115,116}. Although stratified by stage, the data in **Figure 1b**
419 do not take account of the difference in outcomes between stages T1a and T1b, T2a and T2b, and T3a
420 and T3b, to which these factors will contribute. Similarly, the extent to which significantly greater sex
421 differences in stage are related to biological differences, access to healthcare, or delays in diagnosis
422 cannot easily be determined with the available data. Socio-economic factors impacting access to care
423 may drive some of the differences¹¹⁶. For example, it is striking that differences in the rate of MIBC at
424 diagnosis between sexes according to SEER data is greater among African-Americans (30% males vs
425 43% females) versus Caucasians (22% vs 25%). In addition to potential biological drivers of these race
426 differences, access to care is likely a major contributor based on similar data for other cancers¹¹⁷.
427 Social barriers which limit access to care and timely referral are thus important to address. BC
428 disproportionately affects patients with lower socio-economic status and research from Canada
429 suggests that this gap has broadened, particularly among women¹¹⁸. Similar research elsewhere can
430 help to highlight regional and national deficiencies which can stimulate policy and funding changes at
431 a larger level.

432 As part of healthcare delivery, decision support tools (DSTs) may be useful due to their ability to be
433 embedded within practice electronic medical systems so that they are easily accessible during
434 consultation¹¹⁹. They can be automated to draw in background information on smoking status or
435 family history to prompt clinicians to refer for investigation of a certain condition. In an evaluation of
436 a 7-point checklist DST for the assessment of pigmented skin lesions, primary care physicians found
437 such tools easy to use and particularly useful for borderline decision-making¹²⁰. However, widespread
438 use of DSTs relies on the levels of trust placed in the tools, compatibility of the DSTs with specific

439 electronic care systems, and difficulty in usage¹¹⁹. Furthermore, the ability of DSTs to affect change in
440 cancer survival is uncertain and requires ongoing investigation. In the context of haematuria, models
441 have been identified that could be used in primary care to guide referrals, with the potential to identify
442 lower-risk patients with visible haematuria and to stratify individuals who present with non-visible
443 haematuria¹²¹. A recent systematic review identified 13 such models with good discrimination for the
444 diagnosis of bladder or kidney cancer (area under the receiver operating curve, AUROC >0.8), although
445 only 8 had been externally validated; all of the studies had either high or unclear risk of bias¹²¹. The
446 authors concluded that external validations in appropriate populations were required before
447 implementation in primary care¹²¹.

448

449 **Optimising guideline-based care**

450 The establishment and popularisation of guidelines for the referral of women with haematuria are
451 vital to promote standards of care for referral to cystoscopy. Accounting for different age-adjusted
452 cancer risks, some referral guidelines for haematuria differ between men and women¹²². For example,
453 the American Urological Association guidelines give greater weight to non-visible haematuria in men
454 versus women during the 5th and 6th decade of life¹²³. However, the relative paucity of research data
455 to establish these recommendations and the variability of current recommendations between
456 jurisdictions contributes to potential confusion and uncertainty among primary care providers³⁰.

457 Should the standard of care for female patients with urothelial carcinoma treatment differ from male
458 patients? This remains a challenging question, particularly as treatment patterns and practice evolves
459 over time. Some historical data suggest that response to treatment may be inferior for women^{11,15,52}.
460 This includes a meta-analysis suggesting that female sex is associated with poorer cancer-specific
461 survival and inferior responses to BCG. For NMIBC, when evaluated critically, we believe the most
462 reliable and recent data do not support the notion that treatment guidelines should differ between
463 men and women. In metastatic urothelial cancer, men and women appear to have similar treatment
464 outcomes across various studies¹²⁴. Therefore, despite the poorer outcomes reported for female
465 patients with advanced BC, it is not clear that alternate standards of care are warranted.

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

470

471

472 **Harnessing biological differences to improve treatment**

473 A meaningful assessment of the fundamental sex differences in urothelial transformation and
474 subsequent cancer biology is challenging as it transects the complex interplay of sex hormones,
475 environmental exposures, microenvironment, immunology, genetics and genomics, and the
476 microbiome. Nonetheless, this research is critical to identify specific differences which can be
477 translated to clinical care – a call to action which has recently been emphasised¹⁹. However, in an era
478 where molecular classification, personalised medicines and targeted therapies are endlessly sought,
479 this ‘simple’ knowledge may remain years away – with approximately 1 in 4 patients female, many
480 studies are underpowered to answer the question of whether a certain biomarker or classifier or
481 treatment is effective or not in female patients.

482 To address some of these challenges, it is important to design studies accordingly¹⁹. For example, in
483 pre-clinical studies, attention is needed to account for the sex of origin of cell lines and to include
484 studies in both male and female mice. In clinical trials, reporting of results should include sex-based
485 analyses. Furthermore, evaluating prognostic and predictive biomarkers for prognosis and response
486 to therapy according to sex is necessary in correlative analyses of clinical trials. Notably, the
487 recruitment of women into clinical trials is an important pre-requisite for these analyses as this has
488 been a historical challenge. For instance, in a series of well-known NMIBC trials in Spain, only 11% of
489 all recruited patients were women¹²⁵.

490 Biological differences may present unique opportunities to tailor treatment according to sex.
491 Preclinical studies suggest that alterations in sex steroids with AR antagonism may represent a
492 strategy to treat male bladder tumours alone or in combination with immunotherapy. Based on initial
493 pre-clinical studies, AR antagonism may alter immune responsiveness to immunotherapy through
494 alterations in the steroid milieu⁹². Hormonal differences are broadly recognized to impact the immune
495 response¹²⁶, and AR suppressive therapy may improve BC outcomes through a hormonal-mediated
496 modulation of the TME¹²⁷. Furthermore, there is now a significant amount of clinical data to suggest
497 that 5-alpha reductase inhibitors (5ARIs) could decrease the incidence and recurrence rates of low-
498 grade BC in men. With a 2021 meta-analysis suggesting a HR of 0.46 for recurrence for male NMIBC
499 patients receiving 5ARI therapy, prospective clinical trials are warranted. However, these should be
500 adequately powered to avoid false-negatives from short-term evaluation of a long-acting mechanism
501 of action¹²⁸.

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505 **Future directions**

506 Research and advocacy are important to address the sex gaps in clinical care. Improved data collection
507 on the natural history and epidemiologic difference in haematuria between sexes can drive the
508 development of best practices and referral pathways¹⁸. Similarly, understanding the gaps in referral
509 can facilitate targeted campaigns to raise awareness in both the populations at risk and their
510 healthcare providers.

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

515 Clinical research is now emerging to evaluate whether sex-specific treatments are effective, such as
516 suppression of the androgen axis in combination with existing treatments (e.g. the 'BicaBCa' study,
517 NCT05327647) in men. Further studies to target sex-specific strategies to decrease recurrences of
518 NMIBC or progression of MIBC are similarly warranted given existing data¹²⁷, and increased reporting
519 of sex-based analyses in clinical trials is to be welcomed. Importantly, improved awareness of the sex
520 gap is also apparent, as illustrated by a number of presentations at this year's American Society of
521 Clinical Oncology Genitourinary cancers symposium¹²⁹⁻¹³¹.

522

523 **CONCLUSIONS**

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

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882

883

884 **COMPETING INTERESTS**

885 PT reports research funding from AstraZeneca, as well as personal fees as a consultant from Abbvie,
886 Bayer, Knight Pharmaceuticals, Tolmar and TerSera. AW and AB disclose funding received from
887 AstraZeneca for a PhD studentship for AB supervised by AW. AW discloses funding from imCORE for
888 RE-ARM trial analysis. AW acknowledges funding from the RMH/ICR Cancer Research UK RadNet
889 Centre. RTB discloses research funding from Janssen, QED Therapeutics and UroGen Pharma, and
890 consultancy for Nonacus Limited and Cystotech ApS. AC discloses funding from Cancer Research UK,
891 National Institute of Health Research, Prostate Cancer UK, UK Research & Innovation, Elekta AB,
892 honoraria from Bayer PLC, Janssen, AZ, ASTRO, ASCO, Roche, Merck and is Editor in Chief of BMJ
893 Oncology. AC is supported by the NIHR Manchester Biomedical Research Centre.

894

895 **KEY POINTS**

- 896
- 897 • Bladder cancer is a common malignancy with a 3-4 to 1 male preponderance, suggesting
898 relative 'resistance' to the development of the disease in females compared to males.
899 Environmental exposures, hormone signalling, gene expression, immunology and tumour
900 microenvironment likely coalesce to underpin this sexual dimorphism.
 - 901 • Although bladder cancer outcomes are considered to be notably worse for females than
902 males, this finding is not consistent across territories and treatment modalities, suggesting
903 the considerable influence of healthcare system factors on outcomes. Such factors may
904 include diagnostic delays and discrepancies in the appropriate and timely use of guideline-
905 based care.
 - 906 • Addressing healthcare system factors by the implementation of best practices for referral,
907 diagnosis and treatment could provide rapid improvements in outcomes where deficiencies
908 exist.
 - 909 • Nevertheless, the biological phenomena driving the sexual dimorphism in disease incidence
910 are likely to also influence treatment responses, and better understanding of these
911 mechanisms through carefully-designed fundamental research, and preclinical and clinical
912 studies, may reveal sex-specific biomarkers or treatment approaches to benefit all bladder
913 cancer patients.

913

914

915 **TABLE & FIGURE LEGENDS**

916 **Table 1:** Summary of the main characteristics of the consensus classes of muscle invasive bladder
917 cancer (MIBC) (adapted from⁷⁷).

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

920 **Figure 1:** 1-year, 2-year, 3-year, and 4-year bladder cancer survival (%) for adults (aged 15 to 99 years)
921 diagnosed in England 2015 to 2019, followed up to 2020¹⁷. **(a)** Age-standardised and non-age
922 standardised net survival (%) by sex. **(b)** Age-standardised net survival (%) by stage and sex. **NB:** 3-
923 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
924 female patients. Age-standardisation represents a weighted-average of mortality rates for each sex
925 based on the International Classification of Survival Standard.

926 **Figure 2:** Overall survival for patients with T1–T4 bladder cancers treated in England from 2013 to
927 2019, plotted using the Kaplan-Meier method with respect to sex (reproduced with kind permission
928 from John Wiley and Sons¹⁸).

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

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

939

940 **SHORT SUMMARY**

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