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Sex as modifier of survival in patients with advanced urothelial cancer treated with pembrolizumab

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Gender- and sex-based disparities in response to immune-checkpoint inhibitors (ICI) has been reported in a variety of tumor types. Women have different anatomy with recurrent urinary tract infections, a different sex hormonal profile, and intrinsic differences in local and systemic immune systems and urobiome composition. Existing literature data in a pan-cancer context reveal contradictory results, and real-world evidence in urothelial carcinoma (UC) is lacking. This was a real-world, multicenter, international, observational study to determine the sex effects on the clinical outcomes in metastatic urothelial carcinoma (mUC) patients progressing or recurring after platinum-based therapy and treated with pembrolizumab as a part of routine clinical care. A total of 1039 patients, treated from January 1st, 2016 to December 31st, 2023 in 68 cancer centers were included. Our data showed that women with metastatic urothelial carcinoma treated with pembrolizumab had shorter OS than men, with a 13% advantage in the 5-year OS rate for male patients. A deeper understanding of these results may inform sex-stratification in future prospective clinical trials and help develop strategies to reduce the magnitude of the sex disparities observed in urothelial cancer outcomes.

Keywords Immunotherapy, NCT05290038, Pembrolizumab, Sex differences, Urothelial Cancer

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Bladder Cancer (BC) is the 10th most common cancer worldwide, and urothelial carcinoma (UC) is the most prevalent histologic subtype of the upper and lower urinary tracts, accounting for approximately 90% of tumors[1]. Previous and current research indicates sex and gender disparities in the epidemiology and clinical outcomes of bladder UC[2]. The incidence rate is four times higher in men than in women, but women have more aggressive disease, higher risk of recurrence, and lower 5-year survival rate[3–9]. Although the influence of sexual dimorphism on epidemiology and patient survival could depend on sex-specific biological differences, risk profiles, and environmental exposures, the exact underlying mechanism is currently unknown[10,11]. Certainly, women have different anatomy with recurrent urinary tract infections, a different sex hormonal profile, and intrinsic differences in local and systemic immune systems[11,12]. At the same time, men show higher smoking rates and occupational carcinogen exposure, potentially impacting sex disparities in tumor incidence[10,13].

Immune checkpoint inhibitors (ICIs) have expanded the treatment landscape of urothelial carcinoma. ICIs are used as maintenance therapy in metastatic UC (mUC) patients with stability or response to platinum-based chemotherapy, as first-line treatment in combination with enfortumab vedotin, or in the second-line setting after a previous platinum-based chemotherapy[14–18]. Sex-driven disparities in response to immunotherapy and immune-related adverse events (irAEs) have been reported in a variety of tumor types[19,20]. A meta-analysis of randomized clinical trials showed that men treated with ICI used as single therapy experienced heightened survival benefit in several solid tumors compared to women[21]. Nevertheless, the following data showed no significant difference in immunotherapy efficacy between male and female cancer patients[22]. In the MOUSEION-01 study, the meta-analysis of randomized trials on immunotherapies and combinations showed a slight differences in terms of antitumor efficacy between male and female patients, with pooled overall survival (OS) Hazard Ratio (HR) of men treated with single-agent immunotherapy higher than women, and superior benefit in women treated with the immuno-oncology combinations[23].

Several observations could support the existence of a sex imbalance in UC patients treated with immunotherapy. However, in urothelial cancer, the impact of gender on pembrolizumab response was not investigated. Interestingly, recent reports from RNA analyses have revealed sex differences in the urinary microbiome profile of urothelial cancer patients, with a preponderance of Lactobacillus in women and Corynebacterium in men[24]. This differential distribution of microorganisms in the female and male urine could contribute to sex disparity in the host intrinsic physiological immune features associated with the pretreatment tumor microenvironment (TME). Thus, it remains to be elucidated if the dynamic crosstalk between biological, immunological, hormonal, genetic, and epigenetic factors in the context of sex differences, can potentially impact to ICI-response.

The ARON-2 study (ClinicalTrials.gov identifier NCT05290038) was a multicenter, international, retrospective study to collect real-world data on the effectiveness of pembrolizumab in the post-platinum setting[25–30]. In the present study, we present the analysis of clinical outcomes of pembrolizumab according to the patient's sex.

Methods

Study design and population

We retrospectively analyzed clinical data from patients diagnosed at age ≥ 18 years with UC and radiologically confirmed metastatic disease. The study population included patients progressing or recurring after platinum-based therapy and treated with pembrolizumab between January 1st, 2016 and December 31st, 2023 in 68 oncological centers from 21 countries. All included patients had known data on age, gender, tumor histology, Eastern Cooperative Oncology Group-Performance Status (ECOG-PS), sites of metastases, previous surgery and chemotherapy, durations, and response to pembrolizumab according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. Body Mass Index (BMI) was defined as a person's weight in kilograms divided by the square of height in meters.

Clinical and pathological information was extracted, at each participating center, from the patients' medical and pathology reports for clinical use. Physical and laboratory tests, computed tomography (CT), and magnetic resonance imaging (MRI) scans, were performed following standard local procedures.

Patients with missing clinical or outcome data were excluded from the ARON-2 study.

The study protocol was approved on February 17, 2022 by the Ethical Committee of the coordinating center (Marche Region – Italy - No. 2022 39, Study Protocol "ARON 2 Project") and by the Institutional Review Boards of participating centers. The study was conducted according to Good Clinical Practice (GCP) and International Ethical Guidelines for Biomedical Research, and the protocol has been designed with the ethical principles laid down in the Declaration of Helsinki on human experimentation. The informed consent was obtained from the patients, and was waived by the Institutional Review Board of the coordinating center for deceased or untraceable patients.

Study objectives

The primary objective of the present analysis was to assess the clinical outcome mUC patients treated with pembrolizumab according to the patient's sex. The radiological tumor response [progression disease (PD),

stable disease (SD), partial response (PR), complete response (CR)] according to RECIST version 1.1., overall response rate (ORR), progression-free survival (PFS), and OS were collected. OS was calculated from the start of the treatment with pembrolizumab to death for any cause. PFS was defined as the time from the start of pembrolizumab to progression or death from any cause. Duration of response (DoR) is defined as the time from the start of pembrolizumab to disease progression or death in patients who achieved CR or PR. The ORR was calculated as the sum of CR + PR. We considered censored those patients without progression or death at their last follow-up.

Statistical considerations

The analysis of PFS and OS between groups was compared using the Kaplan-Meier method and log-rank test. The median follow-up was calculated with the Kaplan–Meier method. Cox proportional hazard models were used to compare the multivariable effects on patients' survival, and to calculate HRs and 95% confidence intervals (CIs). To better stratify patients according to the number of metastatic sites, a survival receiver operating characteristic (ROC) analysis was exploited to determine potential cut-offs. To assess the potential differences between variables, Fisher's exact test was performed to assess statistically significant associations between dual categorical variables, chi-square test for multiple categorical variables. Were considered statistically significant p values < 0.05.

Results

Patient population

We included 1039 patients treated with pembrolizumab from the ARON-2 dataset; 765 (74%) were males and 274 (26%) were females. The median follow-up time was 24.6 months (95%CI 16.2 – 83.7). Smoking history was more common in males [574/765 (75%) vs. 118/274 (43%), $p < 0.001$]. One hundred sixty seven patients (16%) had ECOG-PS ≥ 2. Pure urothelial carcinoma histology was reported in 831 (80%) of patients; 761 (73%) reported tumors of the bladder and 278 (27%) of the upper urinary tract. Synchronous metastatic disease was reported in 341 patients (33%) and was more frequent in females. Lymph nodes (65%), lung (34%) and bone (29%) were the most frequent metastatic sites. The complete list of patients' characteristics is summarized in Table 1 and Table S1.

Outcomes analysis

The median OS was 14.8 months (95%CI 12.5–16.1) in the overall study population: 15.6 months (95%CI 13.4–17.9) in males and 11.7 months (95%CI 7.9–60.0) in females ($p = 0.021$, Fig. 1). The 5y-OS rate for male patients was 24% vs. 11% for female patients, $p = 0.016$). A total of 555 patients (53.4%) [(392 males (70.6%) and 163 females (29.4%)] had died at the time of data analysis.

Although representing a smaller subset of patients, among aged 18-49y, the median OS was 16.8 months (95%CI 6.0–83.6) in males and 7.5 months (95%CI 2.9–16.3, $p = 0.037$, Fig. 1) in females, while no significant differences were found in patients aged 50-69y (15.9 months, 95%CI 13.2–21.8, vs. 11.3 months, 95%CI 7.5–24.0, $p = 0.313$) as well as in the 541 patients aged ≥ 70y (14.6 months, 95%CI 10.8–80.0, vs. 12.1 months, 95%CI 6.6–60.0, $p = 0.207$) (Figure S1).

No statistically significant differences were found in both current or former smokers (males: 17.0 months, 95%CI 14.8–22.2; females: 15.3 months, 95%CI 8.5–60.0, $p = 0.320$) and non-smokers (males: 13.6 months, 95%CI 10.0–83.6; females: 9.2 months, 95%CI 6.4–13.4, $p = 0.294$).

In patients with an ECOG-PS = 0–1, the median OS was 19.4 months (95%CI 16.0–23.2) in males and 15.0 months (95%CI 10.3–60.0) in females ($p = 0.015$, Fig. 1). Otherwise, no differences were found in patients with an ECOG-PS ≥ 2 (males: 4.3 months, 95%CI 3.2–33.1; females: 3.2 months, 95%CI 1.9–6.0, $p = 0.387$) (Figure S1).

We further stratified patients according to tumor histology. A statistically significant difference in terms of median OS was found in patients with pure urothelial histology (males: 15.6 months, 95%CI 13.2–17.9; females: 11.3 months, 95%CI 6.8–60.0, $p = 0.018$, Fig. 1), but not in patients with mixed histology (males: 15.9 months, 95%CI 7.6–78.8; females: 11.7 months, 95%CI 7.5–19.6, $p = 0.583$) (Figure S1).

In patients with lower tract UC, the median OS was significantly longer in males (15.5 months, 95%CI 13.4–18.5, vs. 10.3 months, 95%CI 6.6–48.6, $p = 0.006$, Fig. 1), while no differences were found in patients with upper tract UC (15.9 months, 95%CI 8.9–22.3, vs. 12.4 months, 95%CI 8.4–60.0, $p = 0.874$) (Figure S1).

The median OS was longer in males with synchronous metastatic disease (14.1 months, 95%CI 10.8–83.6, vs. 8.2 months, 95%CI 5.9–60.0, $p = 0.015$, Fig. 1) but not in subjects with metachronous disease (15.9 months, 95%CI 14.6–21.2, vs. 13.0 months, 95%CI 10.3–20.5, $p = 0.440$) (Figure S1).

When we stratified patients according to the site of metastases, the median OS was longer in males vs. females in patients with metastases to lymph nodes (15.8 months, 95%CI 13.3–20.1 vs. 11.7 months, 95%CI 7.5–15.3, $p = 0.015$, Fig. 2), lung (14.6 months, 95%CI 11.7–83.6 vs. 7.4 months, 95%CI 5.9–40.1, $p = 0.039$, Fig. 2), or liver (10.2 months, 95%CI 6.7–14.9 vs. 4.2 months, 95%CI 3.3–7.5, $p = 0.026$, Fig. 2), while no statistically significant differences were found in patients with bone (7.0 months, 95%CI 5.8–80.0 vs. 6.2 months, 95%CI 3.8–8.5, $p = 0.242$) or brain metastases (5.8 months, 95%CI 3.0–15.3 vs. 2.3 months, 95%CI 0.6–6.9, $p = 0.606$).

The best cut-off for the number of metastatic sites calculated by the ROC curve resulted ≥ 2. Males showed a significantly longer median OS in the subgroup with ≥ 2 sites (10.2 months, 95%CI 8.0–83.6 vs. 6.8 months, 95%CI 4.8–60.0, $p = 0.022$, Fig. 2) but not in those with 1 site of metastasis (21.8 months, 95%CI 16.5–28.0, vs. 16.3 months, 95%CI 10.7–24.3, $p = 0.197$) (Figure S1).

As for the median PFS, it was 5.3 months in the overall study population (95%CI 4.4–83.1) and it was 5.6 months (95%CI 4.4–83.1) in males and 4.7 months (95%CI 3.6–6.5, $p = 0.706$) in females, respectively; 541 males

Characteristics	Overall No. (%)	Males No. (%)	Females No. (%)	*p*-value
Total patients	1039 (100)	765 (100)	274 (100)	-
Median age (IQR)				
18-49y	57 (5)	40 (5)	17 (6)	0.757
50-69y	441 (42)	337 (44)	104 (38)	0.39
≥ 70y	541 (53)	388 (51)	153 (56)	0.48
Current or former smokers				
Yes	692 (67)	574 (75)	118 (43)	**< 0.001**
No	347 (33)	191 (25)	156 (57)	
BMI				
≤ 25 kg/m^2	586 (56)	405 (53)	181 (66)	0.084
> 25 kg/m^2	453 (44)	360 (47)	93 (34)	
ECOG performance status				
0–1	872 (84)	640 (84)	232 (85)	0.846
2–3	167 (16)	125 (16)	42 (15)	
Tumor histology				
Pure urothelial carcinoma	831 (80)	623 (81)	208 (76)	0.391
Minor or mixed variants	208 (20)	142 (19)	66 (24)	
Primary tumor location				
Upper urinary tract	278 (27)	177 (23)	101 (37)	**0.031**
Lower urinary tract	761 (73)	588 (77)	173 (63)	
Metastatic disease				
Synchronous	341 (33)	234 (31)	107 (39)	0.492
Metachronous	698 (67)	531 (69)	167 (61)	
Common sites of metastasis				
Lymph nodes (non-regional)	678 (65)	497 (65)	181 (66)	0.882
Lung	349 (34)	251 (33)	98 (36)	0.656
Bone	297 (29)	224 (29)	73 (27)	0.753
Liver	187 (18)	142 (19)	45 (16)	0.578
Brain	21 (2)	13 (2)	8 (3)	0.651
N sites ≥ 2	555 (53)	409 (53)	146 (53)	1

Table 1. Patient characteristics. Significance values are bold.

(71%) and 195 females (71%) experienced PD during pembrolizumab treatment at the time of data analysis. The 33% of males and 37% of females who progressed during pembrolizumab received further therapies (Table S1).

Response to pembrolizumab

Response to therapy consisted of 8% CR, 22% PR, 24% SD, and 46% PD (ORR = 30%, Table S1). In male patients, we observed 8% CR, 23% PR, 24% SD, and 45% PD (ORR = 31%, Table S1), while females showed 8% CR, 19% PR, 24% SD and 49% PD (ORR = 27%, Table S1). In patients who achieved CR or PR, the median DoR was not significantly different based on sex (males: 13.1 months, 95%CI 10.5–16.8; females: 10.9 months, 95%CI 7.9–14.0, $p = 0.283$, Table S1).

Univariable and multivariable analysis

We performed the univariable and multivariable analysis in the overall study population. At univariable analysis, sex, BMI, ECOG-PS, synchronous metastatic disease, liver, bone, and brain metastases were significantly associated with OS. At multivariable analysis, sex, BMI, ECOG-PS, liver, bone, and brain metastases remained significantly associated with OS (Table 2).

Furthermore, we performed additional multivariable analyses in the three subsets in which the differences in terms of median OS were ≥ 6 months between males and females: age < 50y, lung and liver metastases. We observed that sex was significantly associated with OS at multivariable analysis in both patients with lung (Table S3) or liver metastases (Table S4), but not in patients aged < 50y (Table S2).

Discussion

Gender disparities in urothelial cancer presentation and clinical outcomes have been previously reported[2–6]. Despite the higher incidence of urothelial cancer overall among males, female-gender patients have more advanced-stage tumors at the primary diagnosis, more frequent recurrence or progression, and, overall, poor outcomes[7–9]. Biological and immunological differences underlying sex-driven disparities in urothelial carcinoma are widely investigated, from androgen and estrogen expression to differential mutations, gene

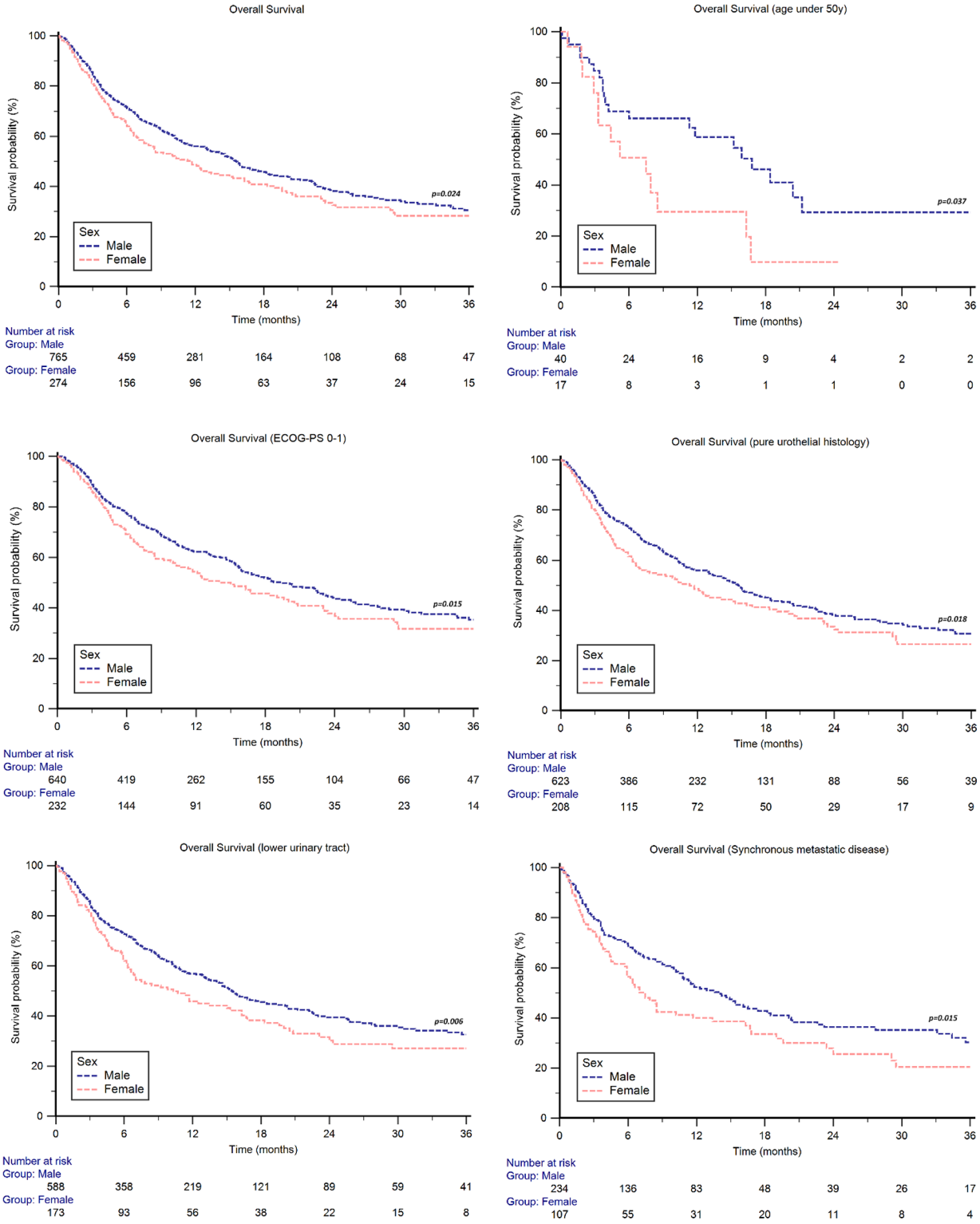

Fig. 1. Overall Survival in patients receiving pembrolizumab stratified by sex and clinic-pathological features.

expression, metabolic enzymes, and urinary microbiome profile[10–13]. These findings emphasize the importance of understanding the factors potentially involved in sex-specific differences in immunotherapy response. Currently, there is a global ongoing debate on whether ICIs have a variable benefit between male and female cancer patients. In a pan-cancer context, despite a trend of inferior response in females, existing literature data showed contradictory results[31]. In the mUC patients, a recent meta-analysis showed the OS benefit of ICI-based combination therapy regardless of sex[32]. In a second systematic review and meta-analysis, females had a comparable median OS to men, and only for the atezolizumab women have a significantly better ORR[33].

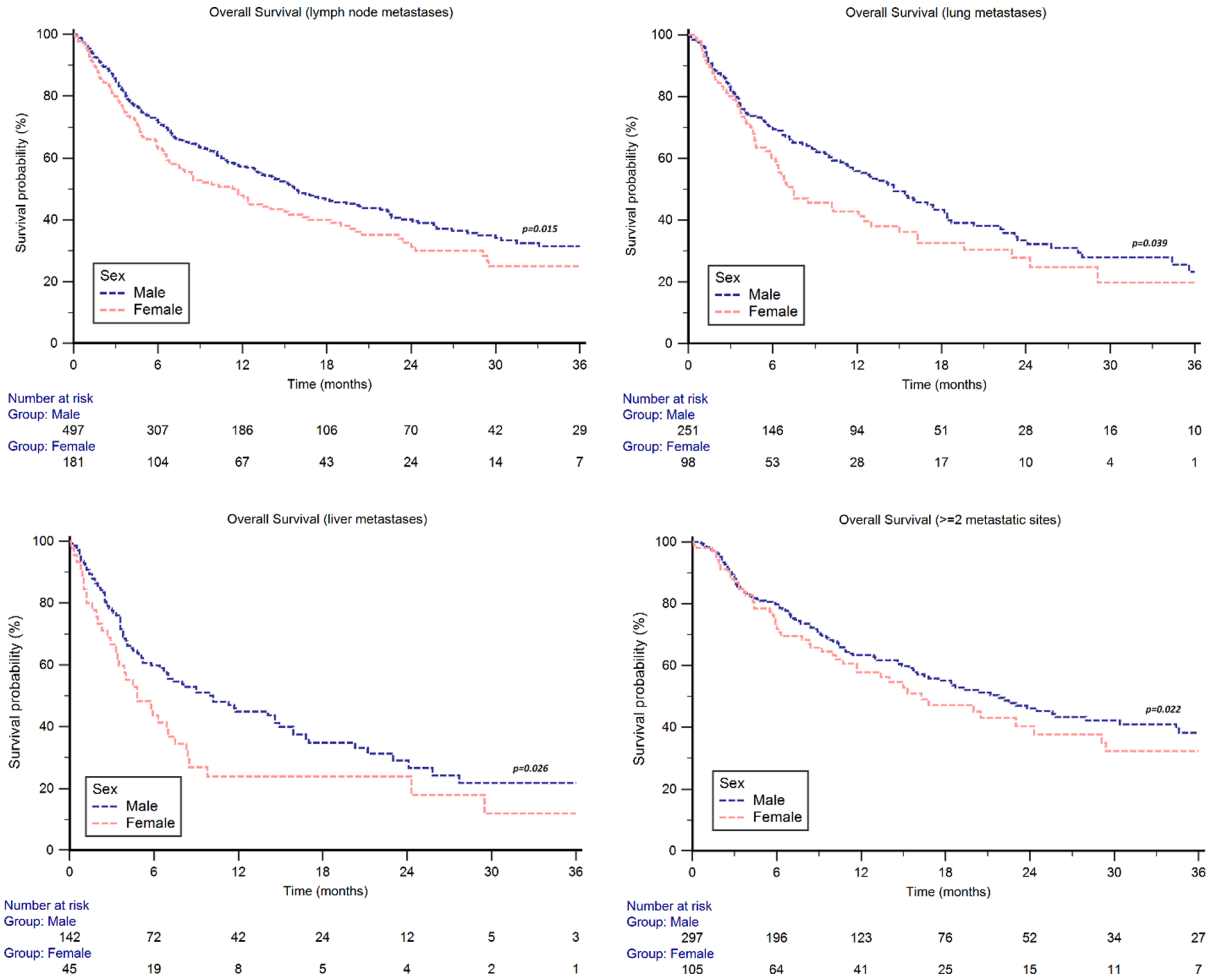

Fig. 2. Overall survival in patients receiving pembrolizumab stratified by sex and type or number of metastatic sites.

	Univariable cox regression		Multivariable cox regression	
Overall survival (overall population)	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
Sex (females vs. males)	1.24 (1.03–1.49)	**0.022**	1.24 (1.03–1.50)	**0.024**
Age < 50y (Y vs. N)	1.20 (0.85–1.69)	0.298		
BMI (> 25 vs. ≤ 25 kg/m^2)	0.73 (0.62–0.87)	**< 0.001**	0.77 (0.65–0.92)	**0.004**
Smokers vs no-smokers	0.85 (0.70–1.01)	0.072		
ECOG PS (≥ 2 vs. 0–1)	3.17 (2.59–3.87)	**< 0.001**	2.87 (2.33–3.52)	**< 0.001**
Histology (mixed vs. pure UC)	1.04 (0.84–1.28)	0.742		
Upper vs Lower urinary tract	1.01 (0.88–1.16)	0.856		
Synchronous metastatic disease (Y vs. N)	1.25 (1.05–1.48)	**0.012**	1.11 (0.93–1.33)	0.250
Lymph node (Y vs. N)	0.85 (0.71–1.02)	0.083		
Lung metastases (Y vs. N)	1.16 (0.97–1.38)	0.097		
Liver metastases (Y vs. N)	1.53 (1.24–1.87)	**< 0.001**	1.58 (1.29–1.94)	**< 0.001**
Bone metastases (Y vs. N)	1.56 (1.32–1.88)	**< 0.001**	1.44 (1.20–1.73)	**< 0.001**
Brain metastases (Y vs. N)	2.32 (1.41–3.82)	**0.001**	2.99 (1.80–4.96)	**< 0.001**

Table 2. Univariable and multivariable analyses in the overall study population. BMI, Body Mass Index; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; Y, yes; N, no.

Importantly, real-world data on the mUC population are lacking.

The objective of the current analysis was to assess whether pembrolizumab effectiveness was different between the sexes in the setting of UC patients who relapsed or progressed after platinum-based chemotherapy. In our large study population of 1039 patients, we observed a significant difference in OS between male and female mUC patients. The median OS was higher in males vs. females, with a 13% advantage in the 5-year OS rate for male patients. Female sex was shown to be an independent negative prognostic factor on multivariable analysis. When we stratified patients according to tumor histology, ECOG-PS, age, synchronous or metachronous metastatic tumor, site, and number of metastases, the median OS was confirmed significantly higher in males vs. females in pure urothelial histology, lower tract UC, patients with ECOG-PS = 0–1, patients aged 18-49y, synchronous metastatic disease, metastases to lymph nodes, lung, liver, and 2 ≥ metastatic sites.

The explanation for this observed disparity between sexes in the clinical outcome of ICI is still speculative and certainly includes the influences of immunological, biological, hormonal, epidemiologic, and environmental factors.

Notably, the use of intravesical Bacillus Calmette-Guérin (BCG) in non–muscle-invasive bladder cancer is among the first examples of success in the manipulation of the immune system to achieve durable anticancer immunity[12]. Following BCG stimulation, previous research reported several differences in sex-related gene expression of T-cell co-repressor molecules, highlighting the potential sex bias in immune responses[34].

More recently, differences in the programmed death-ligand 1 (PD-L1) expression between males and females emerged. Interestingly, PD-L1 expression is partly regulated by estrogen and several X-linked micro-RNAs such as miRNA-221, miRNA-222, miRNA-106b, miRNA-20b, and miRNA-513[35]. Numerous immune response genes showing different expression between the sexes have estrogen receptors (ER) in their promoters[10]. The potential critical role of the estrogens on the ICI response could explain the largest difference in OS in the patients aged 18-49y, where the median OS was 7.5 months in females and 16.8 months in males. Thus, the higher estrogen levels in female patients and a miRNA-based modulation of the PD-1/PD-L1 pathway may represent, at least in part, the hormonal and epigenetic background of sex disparity observed in the ICI response, emphasizing the importance of the complex interplay between biologic factors and immune system[36,37].

At the same time, in bladder cancer, the interplay of androgens, like testosterone, and the immune system, through cytokines production, T cell proliferation, or antibody release, is widely recognized[10]. Supporting these findings, interesting research showed that androgen receptors (ARs) promote the expression of CD24, a glycosyl phosphatidylinositol-linked sialo-glycoprotein expressed on tumor cells, with immunosuppressive properties[38]. The androgen-mediated immune suppression in male UC patients could play a critical role in the pembrolizumab response; the PD-1 inhibitor, through the hormonal influence on the tumor microenvironment, could make the tumors more responsive to ICI.

Furthermore, according to the increased interest in the microbiome composition and effectiveness of anti-PD1/PD-L1 therapy, sex-related urinary microbiome differences are emerging in UC patients. An early study using next-generation sequencing of 16 S rRNA in urine samples showed that the "urobiome" was predominated by Lactobacillales in females and by Corynebacterium species in males, paving the way to the study of the urobiome as a variable in the sexual dimorphisms of the ICI response[39].

Several studies have also assessed the role of concomitant 'immune-modulatory' medications including proton pump inhibitors (PPI), antibiotics, and corticosteroids, on the efficacy of ICIs in patients with advanced UC. These data showed that baseline PPI use was significantly associated with shorter survival in patients with bladder UC (BUC) than upper-tract UC (UTUC), probably through changes in the gut microbiome. Interestingly, in our female study population, the primary cancer site was most frequently BUC[40].

The current study has several strengths, particularly the large sample size. To our knowledge, it is the first real-world study that investigates the influence of sex on ICI outcome in mUC patients, showing the female sex as a negative prognostic factor in the overall population.

We also acknowledge some limitations of our study, mainly due to its retrospective nature. Furthermore, in our study population, ORR was not affected by sex, and in patients who achieved CR or PR, the median DoR was also not significantly different. Although our primary objective was to assess the OS, and it is recognized that conventional RECIST-based endpoints, including ORR, cannot fully characterize the clinical benefit of immunotherapy, the weak associations between ORR, DoR and median OS will need to be further investigated in the future.

Finally, the relationship between sex and response to immunotherapy is complex, and it is probably influenced by environmental factors or genetic/epigenetic events potentially altering the TME, the immune response, immune cell composition, and hormonal axis regulation, further influencing the response to ICI[41,42].

A further understanding of sex-based differences in tumor immune responses may represent a strong rationale to consider sex stratification in future prospective clinical trials.

Conclusion

Our real-world study showed that mUC women progressing or recurring after platinum-based therapy and treated with pembrolizumab had shorter OS than males. Sex female was an independent negative prognostic factor in the overall population and in both patients with lung or liver metastases.

Further investigation is required to better define the relevance and clinical application of these findings, enhancing the opportunities for personalized treatment of mUC patients.

Data availability

"The data that support the findings of this study are available from the corresponding author upon reasonable request."

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Author contributions

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Declarations

Competing interests

Dr. Buti reported receiving personal fees from BMS, Pfizer, MSD, Ipsen, Roche, Eli Lilly, AstraZeneca, Pierr e-Fabre, Novartis, Merck, Gentili, and Astellas outside the submitted work; Dr. Fiala reported receiving personal fees from Novartis, Janssen, Merck, BMS, MSD, Pierre Fabre and Pfizer outside the submitted work; Dr. Incorvaia reported receiving personal fees from Bristol Myers Squibb and IPSEN outside the submitted work; Dr. Massari reported receiving personal fees from Advanced Accelerator Applications, Astellas, Astra Zeneca, Bayer, BMS, Janssen, Ipsen, MSD, Pfizer outside the submitted work. The other authors have no competing interests to declare.

Additional information

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