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Gender disparities in clinical outcomes of urothelial carcinoma linked to X chromosome gene *KDM6A* mutation

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ABSTRACT

Objective *KDM6A*, a representative tumour suppressor gene with sex bias, is frequently altered in urothelial carcinoma (UC). The specific impacts of *KDM6A* mutations on gender-based clinical outcomes in UC remain poorly understood.

Methods and analysis We enrolled 2438 patients with UC from seven independent real-world cohorts possessing comprehensive clinical and genomic data. Point mutations and homozygous deletions of *KDM6A* are categorised as *KDM6A*^{Mut}. We assessed the correlation between gender disparities in relation to *KDM6A* status and clinical outcomes, as well as genomic and immunological profiles.

Results *KDM6A* mutations were identified in 679 of the 2306 patients with UC (29.4%), with 505 of 1768 (28.6%) in men and 174 of 538 (32.3%) in women. *KDM6A* mutations correlated with enhanced overall survival exclusively in male patients but were linked to improved outcomes following adjuvant chemotherapy only in female patients. Concerning immunotherapeutic responses, *KDM6A*^{Mut} male patients displayed the most favourable clinical outcomes, whereas *KDM6A*^{Mut} female patients demonstrated the least favourable outcomes. Independent of gender variations, *KDM6A*^{Mut} patients exhibited heightened androgen receptor and diminished oestrogen receptor 1 filtered regulon activity. Additionally, *KDM6A*^{Mut} male patients showed increased infiltration of T cells, cytotoxic T cells and NK cells with enriched neoantigens, in contrast to *KDM6A*^{Mut} female patients who manifested a more pronounced angiogenesis signature.

Conclusion Our findings offer preliminary clinical evidence accentuating *KDM6A* alterations as a promising prognostic and predictive biomarker while elucidating the gender disparities observed in patients with UC.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Urothelial carcinoma (UC) has notable gender disparities in both incidence and outcomes. *KDM6A*, located on the X chromosome, is mutated in close to 30% of cases with UC and may exhibit gender-based associations.

WHAT THIS STUDY ADDS

⇒ Our findings reveal that male patients with *KDM6A* mutations (*KDM6A*^{Mut}) exhibit extended overall survival and enhanced responsiveness to immunotherapy. Conversely, *KDM6A*^{Mut} female patients derive greater benefits from chemotherapy but demonstrate a suboptimal response to immunotherapy. These insights potentially elucidate the gender disparities observed in UC.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study underscores the significance of integrating gender-specific genetic insights into oncological interventions, as it can lead to more efficacious treatment outcomes. This may help in refining cancer treatment approaches to ensure they are optimised for both genders.

INTRODUCTION

Urothelial carcinoma (UC) is the sixth most common cancer worldwide, and the fourth most prevalent among men. Each year, around 600 000 new cases are diagnosed.[1] Even with the rigorous application of standard-of-care treatments such as surgical resection, chemotherapy and immunotherapy using anti-programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) agents, patients diagnosed with UC often face poor prognoses.[2] A pronounced gender disparity is observed in UC's incidence and outcomes; men have a threefold higher incidence rate but exhibit more favourable prognostic outcomes compared with women.[3] Recent studies have shown that these disparities extend to epigenetic factors, immune microenvironments and even therapeutic responses.[4] Leveraging these gender disparities in crafting treatment strategies for UC is both urgent and paramount.

Numerous comprehensive molecular studies on UC have consistently identified inactivating alterations in chromatin modifier genes, resulting in unique epigenetic states.[5] Lysine demethylase 6A (KDM6A, also known as UTX) belongs to the KDM6

family of histone H3 lysine 27 (H3K27) demethylases and acts as a component of the COMPASS complex to control gene activation. It was first reported in 1998 and has established a direct correlation between the expression pattern of KDM6A and the copy number of the X chromosome, reaffirming its identity as an X chromosome inactivation (XCI) escape gene.[6 7] Since its identification as an H3K27 demethylase in 2007, *KDM6A*'s vital contributions to the regulation of chromatin structure, cell differentiation, embryonic development and cancer have been well-documented in numerous studies.[8 9] *KDM6A* mutations have been found across a spectrum of cancers, including leukemias, lymphomas, oesophageal, gastric, and notably, UC.[10] In fact, UC presents the highest frequency of *KDM6A* mutation, affecting approximately 26% of muscle-invasive bladder cancer (MIBC) and 38% of upper tract UC,[5 11 12] potentially leading to distinct epigenetic shifts within UC.[13 14] *KDM6A* displays a pronounced gender bias. For instance, in T-cell acute lymphoblastic leukaemia, *KDM6A* mutations predominantly arise in male patients but are exempt from X-inactivation in female T cells.[15 16] Within bladder cancer, the combined effects of *KDM6A* loss and FGFR3 activation suppress luminal gene expression, which typically exhibits gender differences.[17] Although gender-biased *KDM6A* mutations in UC have been sporadically reported, the understanding of their impact on clinical outcomes and the mechanisms driving them remains insufficient.

In the present study, we investigate the gender disparities associated with *KDM6A* mutation status on UC's diverse clinical outcomes, encompassing overall survival (OS), responsiveness to platinum-based chemotherapy and immunotherapy. We further probe the signalling pathways influenced by sex hormones to elucidate the genomic and immunological characteristics tied to *KDM6A*-mutant UC, with a particular focus on gender-based variations.

MATERIALS AND METHODS

Patient inclusion

The FUSCC cohort, authorised by the Clinical Research Ethics Committee of Fudan University Shanghai Cancer Center (FUSCC), comprises 101 patients with UC. These individuals underwent radical cystectomy (RC), diagnostic biopsy or transurethral resection of bladder tumour, with genomic data sourced from the FUSCC-UC panel. In contrast, the ZSHS cohort, approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University (ZSHS), consists of 132 patients. All were diagnosed with MIBC and underwent RC. The clinicopathological characteristics of these patients are detailed in table 1.

Public data sets

The Memorial Sloan Kettering Cancer Center (MSKCC) cohort[18] comprises 1218 pathologically diagnosed cases with UC, with survival data available for 1146 of these cases. The Cancer Genome Atlas (TCGA) cohort[5] includes 391 cases with MIBC with DNA sequencing, mRNA expression and clinical status data. The IMvigor210 cohort[19] has 274 patients diagnosed with metastatic UC (mUC) treated with atezolizumab. The Urothelial Cancer - Genomic Analysis to Improve Patient Outcomes and Research (UC-GENOME) cohort[20] includes 123 patients with mUC who received immunotherapy, and survival data are accessible for 109 of these individuals. Lastly, the Memorial Sloan Kettering Cancer Center - IMPACT (MSKCC-IMPACT) cohort[21] encompasses 199 patients with UC treated with immunotherapy. The clinicopathological traits of patients across these datasets are summarised in online supplemental table 1. The specific inclusion criteria for these cohorts are presented in online supplemental figure 1. In total, our study enrolled 2438 patients with UC from two local and five independent public cohorts, all of whom had sufficient clinical and genomic data.

The clinical response to the immune checkpoint inhibitor (ICI) was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1 for the FUSCC, IMvigor210 and UC-GENOME cohorts.[22] To further understand the clinical implications of *KDM6A* in adjuvant chemotherapy, we conducted a pooled analysis of AJCC stage II and III patients who received chemotherapy across the ZSHS, MSKCC and TCGA cohorts. For data sourcing, mutation annotation files, copy number alteration (CNA) data and associated clinicopathological data for the MSKCC, TCGA, UC-GENOME and MSKCC-IMPACT cohorts were procured from http://www.cbioportal.org.[23] Meanwhile, clinical and genomic data from the IMvigor210 trial were accessed through the IMvigor210CoreBiologies R package from http://research-pub.gene.com/IMvigor210CoreBiologies.

Evaluation of *KDM6A* status

In the MSKCC and TCGA cohorts, $KDM6A^{Mut}$ refers to both point mutations and homozygous deletions of *KDM6A*. For the FUSCC, IMvigor210, UC-GENOME and MSKCC-IMPACT cohorts, since no patients underwent CNA testing for *KDM6A*, only point mutations of *KDM6A* are labelled as $KDM6A^{Mut}$, as detailed in online supplemental figure 2. Considering that roughly one-third of patients displayed a *KDM6A* alteration (online supplemental table 1) and that in the TCGA cohort, point mutations of *KDM6A* significantly diminished its transcriptomic expression (online supplemental figure 3A, Mann-Whitney U test, $p<0.001$), the initial 33% of patients exhibiting low KDM6A expression as assessed by immunohistochemistry (IHC) assay were categorised as $KDM6A^{Mut}$ in the ZSHS cohort. Representative images are available in online supplemental figure 3B.

IHC assay

IHC staining was performed on tissue microarrays (TMAs) derived from formalin-fixed, paraffin-embedded specimens. The process by which the TMA was prepared is detailed in a prior publication.[24] Digital scans of all

Table 1 Clinical and demographic characteristics of the patients at baseline

Cohort	FUSCC cohort (*n* = 101)		ZSHS cohort (*n* = 132)	
Gender	Male	Female	Male	Female
	82	19	108	24
Age				
Median age, years (range)	64 (33–79)	67 (30–74)	62 (30–82)	64 (52–78)
Distribution, no. (%)				
<65	43 (52.4)	9 (47.4)	61 (56.5)	13 (54.2)
≥65	39 (47.6)	10 (52.6)	47 (43.5)	11 (45.8)
Median OS, months (range)	Not reached (0.0–201.7)	55.8 (4.5–62.9)	90.0 (1.0–161.0)	46.0 (1.0–146.0)
AJCC stage, no. (%)				
I	21 (25.6)	1 (5.3)	0 (0.0)	0 (0.0)
II	15 (18.3)	3 (15.8)	66 (61.1)	16 (66.7)
III	22 (26.8)	7 (36.8)	42 (38.9)	8 (33.3)
IV	24 (29.3)	8 (42.1)	0 (0.0)	0 (0.0)
Histology, no. (%)				
UC	38 (46.3)	13 (63.4)	84 (77.8)	16 (66.7)
UC with squamous	8 (9.8)	2 (10.5)	5 (4.6)	3 (12.5)
UC with glandular	5 (6.1)	0 (0.0)	1 (0.9)	0 (0.0)
UC with micropapillary	29 (35.4)	4 (21.1)	18 (16.7)	5 (20.8)
UC with other variants	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
KDM6A mutation status, no. (%)				
KDM6A^{Mut}	23 (28.0)	4 (21.1)	NA	
KDM6A^{WT}	59 (72.0)	15 (78.9)		
KDM6A IHC status, no. (%)				
KDM6A^{Mut}	NA		37 (34.3)	7 (29.2)
KDM6A^{WT}			71 (65.7)	17 (70.8)

AJCC, American Joint Committee on Cancer; FUSCC, Fudan University Shanghai Cancer Centre; IHC, immunohistochemistry; Mut, mutation; NA, not available; OS, overall survival; UC, urothelial carcinoma; WT, wild type; ZSHS, Zhongshan Hospital, Fudan University.

slides were obtained using the NanoZoomer-XR (Hamamatsu) and subsequently analysed with Image Pro Plus 6.0 software. Two independent pathologists, blinded to patient data, evaluated the positively stained cells and scored all samples. Cells were examined and quantified under a 200× magnification. The IHC score, derived from staining intensity and the proportion of positive cells, ranged between 0 and 300, with the final value being the average of the two pathologists' scores.

For the ZSHS cohort, the expression status of *KDM6A* was evaluated using the IHC for *KDM6A* (Abcam, ab235989). Given the recognition of PD-L1 IHC staining by the United States Food and Drug Administration (FDA) as a supplementary diagnostic tool for assessing PD-L1 expression levels in patients deliberating immunotherapy, we used the DAKO 22-C3 and DAKO 28-8 assays for the FUSCC cohort. Patients with a combined positive score greater than 10 using the 22-C3 assay or a tumour positive score exceeding 1% with the 28-8 assay were identified as PD-L1 positive.[25]

Processing of genomic and transcriptomic data

For the genomic data, we used Maftools to illustrate the mutation sites of *KDM6A* in both male and female patients.[26] A tumour mutation burden (TMB) surpassing 10 mutations per megabase was categorised as high TMB in line with FDA guidelines.[27] For transcriptomic analysis, gene set enrichment analysis (GSEA) was undertaken to assess the immunogenomic characteristics.[28] Gene sets representing the regulon activity of the androgen receptor (AR) and oestrogen receptor 1 (ESR1) were sourced from Shi *et al*.[29] Within the TCGA cohort, the microenvironment cell populations-counter tool was harnessed to gauge immune infiltration in clinical specimens based on their transcriptomic data.[30] Concurrently, the single sample gene set enrichment analysis (ssGSEA)[31] was also employed to simulate the assessment of tertiary lymphoid structures (TLSs) and the angiogenesis process in these samples (online supplemental table 2). Furthermore, we incorporated the SOPRANO algorithm on the genomic profiles of our patient samples.[32] SOPRANO calculates

the trinucleotide context-corrected dN/dS both within and outside designated genomic regions. The immune dN/dS and immune escape status, as determined by SOPRANO, were intended to showcase immune-specific selection during the interplay between tumour cells and their adjacent microenvironments. All relevant data were extracted from the supplementary data accompanying the algorithm.

Statistical analysis

For survival analysis, the Kaplan-Meier approach combined with the Log-rank test was employed to affirm *KDM6A*^{Mut} as a distinct prognostic factor among male and female patients. The incidence comparison between the two groups was accomplished via the χ^2 test or Fisher's exact test. For continuous variables, the Mann-Whitney U test and the Kruskal-Wallis test were executed to contrast distributions among two or more predefined subgroups. Outcomes are presented as median values alongside their IQRs. GSEA was conducted using GSEA software V.4.0.2 (Broad Institute, Massachusetts, USA).[33] In this study, a p value <0.05 was deemed statistically significant. All statistical evaluations were executed using IBM SPSS Statistics V.22.0 and R V.3.6.3 (http://www.rproject.org/).

Patient and public involvement statement

This investigation incorporated 2438 patients with UC across seven independent public cohorts, all of whom had sufficient clinical and genomic data. As the research approach was retrospective in nature, patient involvement in the study's design was deemed unnecessary. Therefore, patients did not play a role in shaping the research question, collecting and analysing data, interpreting findings or manuscript preparation. Our research results will be disseminated to the wider public via press releases, social media channels, presentations at global conferences, reports furnished to pertinent governmental bodies and scholarly associations.

RESULTS

KDM6A mutation is associated with superior OS in male patients with UC

KDM6A mutations were detected in 679 out of 2306 (29.4%) patients with UC from six cohorts with genomic data, with 505 out of 1768 (28.6%) occurring in men and 174 out of 538 (32.3%) in women (online supplemental figure 2). Among all patients in both ZSHS and MSKCC cohorts, *KDM6A*^{Mut} patients exhibited improved survival compared with *KDM6A*^{WT} counterparts. Specifically, in the ZSHS cohort, the median OS for *KDM6A*^{Mut} patients was 142.0 months, notably longer than the 61.0 months observed for *KDM6A*^{WT} patients (figure 1A left, Log-rank p=0.005). Similarly, in the MSKCC cohort, the median OS for *KDM6A*^{Mut} patients reached 95.6 months, slightly

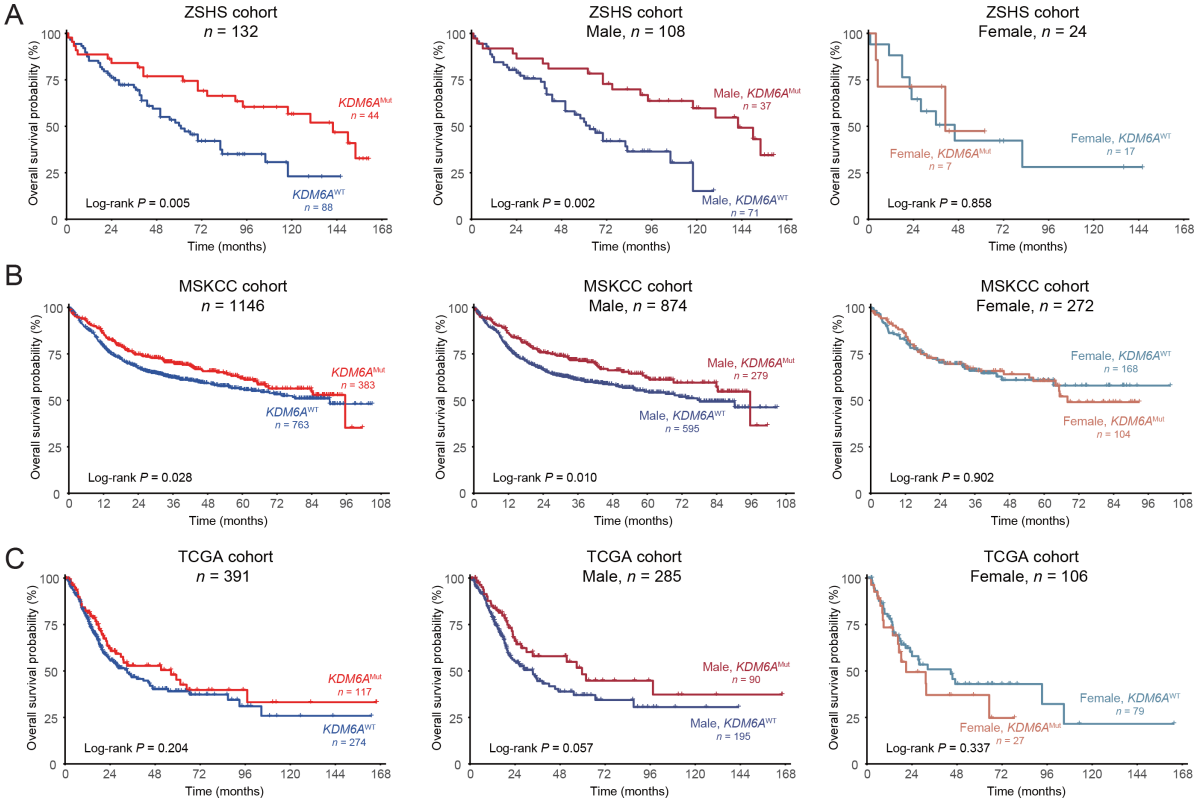

Figure 1 *KDM6A* mutation is associated with superior OS in male patients with UC. (A–C) Kaplan-Meier analysis of overall survival between *KDM6A*^{Mut} and *KDM6A*^{WT} patients in all (left), male patients (middle) and female patients (right) in the ZSHS (A), MSKCC (B) and TCGA (C) cohorts. ZSHS, Zhongshan Hospital, Fudan University; MSKCC, Memorial Sloan Kettering Cancer Center; TCGA, The Cancer Genome Atlas; Mut, mutation; OS, overall survival; UC, urothelial carcinoma; WT, wild type.

exceeding the 90.2 months observed for *KDM6A*^{WT} patients (figure 1B left, Log-rank p=0.028). Moreover, in the TCGA cohort, the median OS was 56.4 months for *KDM6A*^{Mut} patients and 33.0 months for *KDM6A*^{Mut} patients, though statistical significance was absent (figure 1C left, Log-rank p=0.204).

Remarkably, *KDM6A* mutations consistently exhibited a favourable impact on OS in male patients across three cohorts. In the ZSHS cohort, male patients with *KDM6A* mutations displayed a median OS of 142.0 months, contrasting with 63.0 months for wild-type individuals (figure 1A middle, Log-rank p=0.002). Within the MSKCC cohort, the median OS was 95.6 months for male patients with *KDM6A* mutations versus 78.1 months for those without (figure 1B middle, Log-rank p=0.010). In the TCGA cohort, male patients with *KDM6A* mutations displayed a median OS of 59.3 months, compared with 33.0 months for wild-type individuals (figure 1C middle, Log-rank p=0.057). However, in female patients across the same cohorts, no substantial differences in survival were observed based on *KDM6A* mutation status (figure 1A–C right).

KDM6A mutation and improved chemotherapy outcomes in female patients with UC

Given the limited sample size of female patients with UC undergoing chemotherapy, a pooled analysis encompassing the ZSHS, MSKCC and TCGA cohorts was conducted. Generally, patients with *KDM6A* mutations demonstrated a pattern of prolonged OS subsequent to chemotherapy (figure 2A). The median OS for *KDM6A*^{Mut} patients was 130.0 months compared with 61.0 months for wild-type individuals, although the difference did not reach statistical significance (Log-rank p=0.064). Remarkably, *KDM6A* mutations did not exhibit substantial clinical associations among male patients who received chemotherapy (figure 2B). Conversely, *KDM6A*^{Mut} women did not reach the median OS, whereas *KDM6A*^{WT} women showed an OS of 82 months (figure 2C, Log-rank p=0.026).

Gender-specific differences in immunotherapeutic response attributed to *KDM6A* mutation in UC

We further explored if the *KDM6A* alteration might explain gender differences in immunotherapy outcomes among patients with UC. In both the IMvigor210 and UC-GENOME cohorts, the combination of *KDM6A* mutation and gender exhibited notable stratifying potential (figure 3A,B. IMvigor210 cohort, Log-rank p=0.060; UC-GENOME cohort, Log-rank p=0.009). In the IMvigor210 cohort, male patients harbouring *KDM6A* mutations did not reach median OS compared with 7.1 months for female patients with the same mutations (figure 3A, Log-rank p=0.039). Additionally, the response rate was notably higher in *KDM6A*^{Mut} male patients at 45.7% compared with 0.0% in *KDM6A*^{Mut} female patients. In the UC-GENOME cohort, *KDM6A*^{Mut} male patients exhibited a median OS of 93.7 months, significantly higher than the 34.5 months for female patients (figure 3B, Log-rank p=0.004). Furthermore, the response rate was notably elevated in *KDM6A*^{Mut} male patients at 56.3% compared with 20.0% in *KDM6A*^{Mut} female patients. Moreover, within the MSKCC-IMPACT cohort, male patients with *KDM6A* mutations consistently demonstrated extended OS compared with their *KDM6A*^{WT} counterparts. The median OS for *KDM6A*^{Mut} male patients was not reached, while it was 14.0 months for *KDM6A*^{WT} male patients, although the difference did not reach statistical significance (figure 3C, Log-rank p=0.051). This implies that the *KDM6A* mutation consistently predicts positive clinical outcomes in men, whereas in women, it may indicate poor immunotherapy outcomes.

To further substantiate this observation, we examined the hypothesis in the local FUSCC cohort. Of the 27 *KDM6A*^{Mut} patients with UC, 23 were men and 4 were women (figure 3D). All female patients underwent immunotherapy but displayed notably poorer clinical outcomes compared with their male

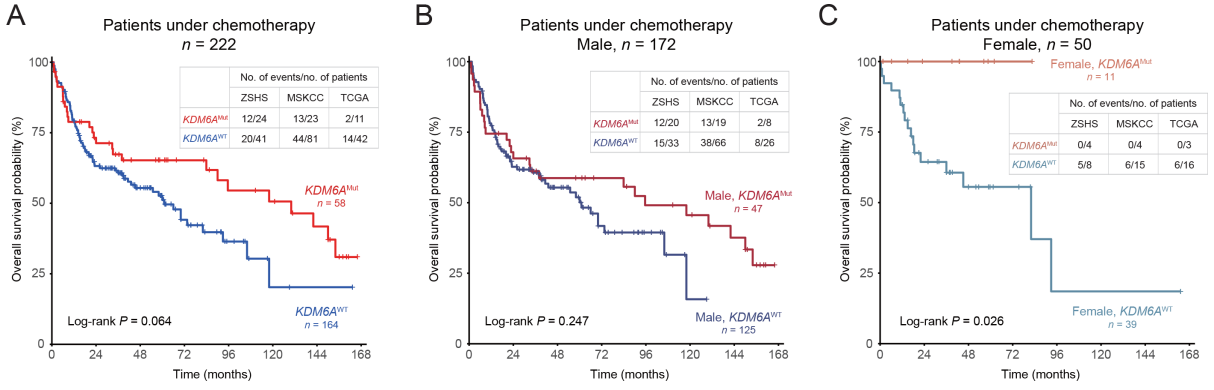

Figure 2 *KDM6A* mutation is associated with better outcome from ACT in female patients with UC. (A–C) Kaplan-Meier analyses of overall survival between *KDM6A*^{Mut} and *KDM6A*^{WT} patients treated with chemotherapy in all (A), male patients (B) and female patients (C) in the pooled analyses of the ZSHS, MSKCC and TCGA cohorts. ZSHS, Zhongshan Hospital, Fudan University; MSKCC, Memorial Sloan Kettering Cancer Center; TCGA, The Cancer Genome Atlas; Mut, mutation; UC, urothelial carcinoma; WT, wild type.

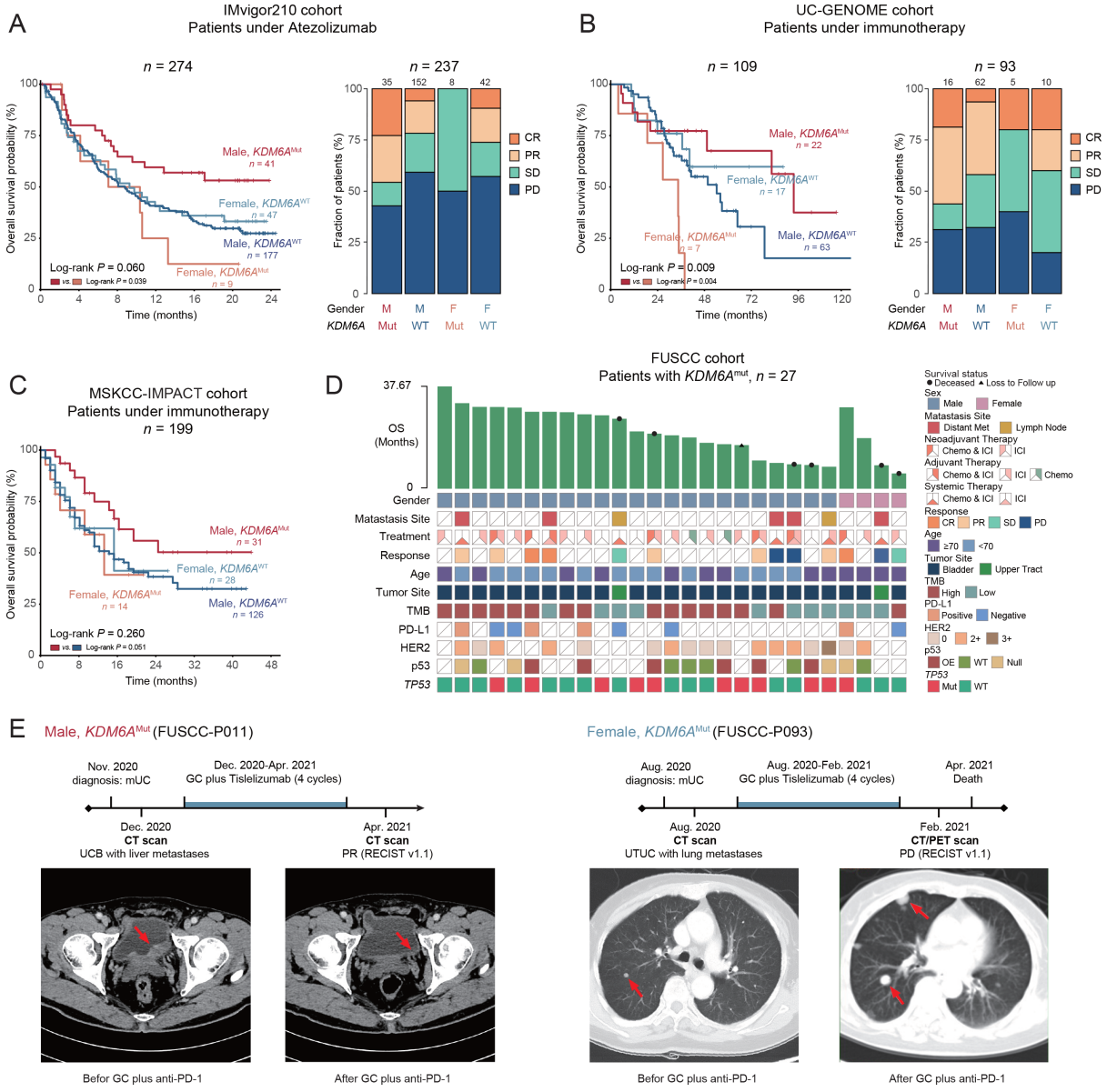

Figure 3 *KDM6A* mutation contributes to gender-specific differences in immunotherapeutic response in UC. (A) Kaplan-Meier analyses of overall survival for patients with different *KDM6A* status and gender treated by atezolizumab (left) and responses to atezolizumab (right) in the IMvigor210 cohort. (B) Kaplan-Meier analyses of overall survival for patients of different *KDM6A* status and gender receiving immunotherapy (left) and responses to immunotherapy (right) in the UC-GENOME cohort. (C) Kaplan-Meier analyses of overall survival for patients of different *KDM6A* and gender status receiving immunotherapy in the MSKCC-IMPACT cohort. (D) Clinicopathological characteristics of *KDM6A*^{Mut} patients in the FUSCC cohort. (E) Serial computational tomography imaging of two *KDM6A*^{Mut} patients with mUC treated with GC plus Tislelizumab in the FUSCC cohort. The red arrows highlight the target lesions and their evolution from baseline to completion of the 4-cycle chemo-immunotherapy. UC-GENOME, Urothelial Cancer - Genomic Analysis to Improve Patient Outcomes and Research; MSKCC-IMPACT, Memorial Sloan Kettering Cancer Center - IMPACT; FUSCC, Fudan University Shanghai Cancer Center; CR, complete response; mUC, metastatic urothelial carcinoma; OE, overexpression; PD, progressive disease; PET, positron emission tomography; PR, partial response; SD, stable disease; UCB, urothelial carcinoma of bladder; UTUC, upper tract urothelial carcinoma; WT, wild type; M, Mut, Male, *KDM6A*^{Mut}; M, WT, Male, *KDM6A*^{WT}; F, Mut, Female, *KDM6A*^{Mut}; F, WT, Female, *KDM6A*^{WT}.

counterparts. *KDM6A*^{Mut} men did not reach the median OS, whereas *KDM6A*^{Mut} women showed an OS of 8.6 months in FUSCC cohort (online supplemental figure 4, Log-rank P = 0.053). For example, two patients, FUSCC-P011 (Male, KDM6AMut) and FUSCC-P093 (Female, KDM6AMut), both diagnosed with mUC, received Tislelizumab, an anti-PD-1 agent. After four treatment cycles, the male patient demonstrated significant remission (RECIST: PR), while the female patient showed disease progression (RECIST: PD) and experienced mortality event within two months (figure 3E).

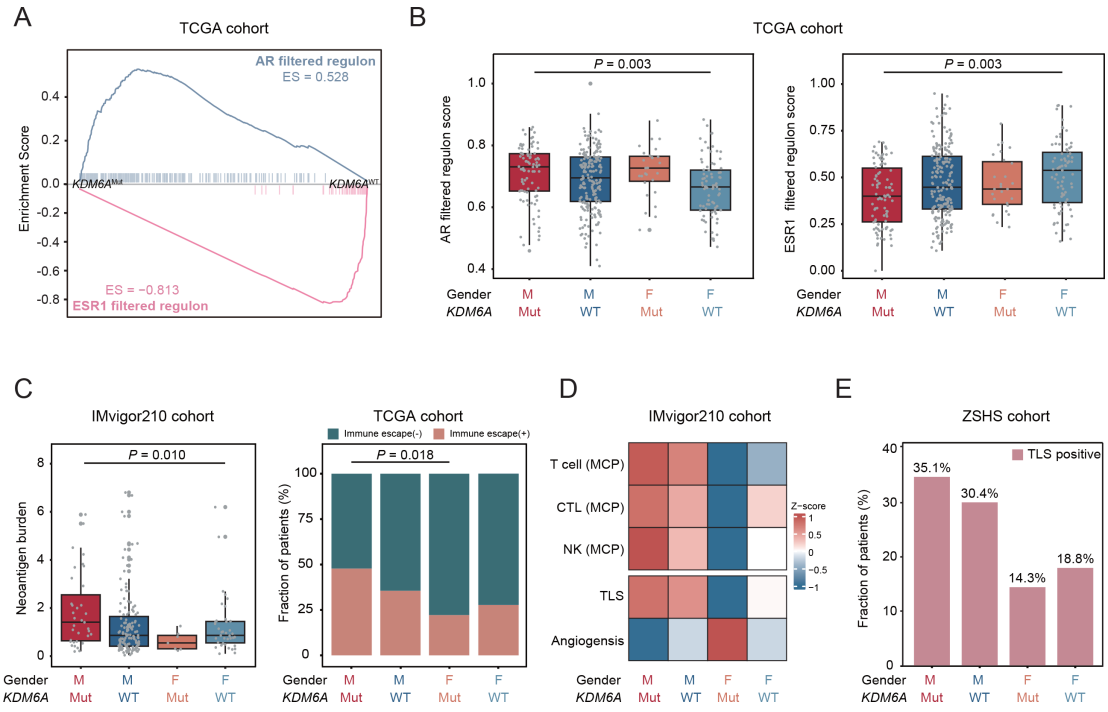

Figure 4 *KDM6A* mutation impacts immune response through Interplay with sex hormone receptor signalling in UC. (A) GSEA plot demonstrating AR and ESR1 enrichment score of *KDM6A*^{Mut} patients compared with *KDM6A*^{WT} patients in TCGA cohort. (B) Boxplot depicting the correlation between AR filtered regulon score (left), ESR1 filtered regulon score (right) and *KDM6A* status in TCGA cohort stratified by gender. (C) Boxplot and bar plot depicting the association between *KDM6A* status and Tumour neoantigen burden in IMvigor210 cohort (left), immune escape status defined by SOPRANO in TCGA cohort stratified by gender. (D) Heatmap illustrating immune effector cells, TLS and Angiogenesis signatures of IMvigor210 cohort to detect the correlation between *KDM6A* status and immune microenvironment stratified gender. (E) Bar plot illustrating different distribution of TLS presence in patients with different *KDM6A* and gender status in ZSHS cohort. TCGA, The Cancer Genome Atlas; ZSHS, Zhongshan Hospital, Fudan University; AR, androgen receptor; CTL, cytotoxic T lymphocyte; ESR1, oestrogen receptor 1; GSEA, gene set enrichment analysis; TLS, tertiary lymphoid structure; M, Mut, Male, *KDM6A*^{Mut}; M, WT, Male, *KDM6A*^{WT}; F, Mut, Female, *KDM6A*^{Mut}; F, WT, Female, *KDM6A*^{WT}.

Impact of *KDM6A* mutation on immune response via interaction with sex hormone receptor signalling in UC

We sought to explore the association between *KDM6A*^{Mut} and biological dysfunctions related to sexual or immunological discrepancies in UC. Intriguingly, GSEA disclosed that in *KDM6A*^{Mut} samples, the AR filtered regulon pathway was significantly upregulated (figure 4A, ES=0.528; online supplemental figure 5, Mann-Whitney U test, p=0.003), while the ESR1 filtered regulon pathway was conversely downregulated (figure 4A, ES=−0.813; online supplemental figure 5, Mann-Whitney U test, p=0.003). Notably, within a gender-specific context, *KDM6A*^{Mut} patients consistently exhibited higher AR and lower ESR1 filtered regulon activity, irrespective of their gender (figure 4B, AR: Kruskal-Wallis test, p=0.003; ESR1: Kruskal-Wallis test, p=0.003).

Regarding immunogenomic characteristics, *KDM6A*^{Mut} male patients in the IMvigor210 cohort were notably enriched for neoantigens (figure 4C, Kruskal-Wallis test, p=0.002). Concurrently, immune escape status, as defined by SOPRANO,[32] was more prevalent in *KDM6A*^{Mut} male patients (figure 4C, χ^2 test, p=0.018), which implies that in addition to the activity of hormone receptors, the intrinsically different genomic contexts between male and female *KDM6A*^{Mut} patients might also have contributed to the different outcomes from immunotherapy. Concerning immune cell infiltration, *KDM6A*^{Mut} male patients exhibited elevated infiltration levels of T cells, cytotoxic T cells and NK cells. Transcriptomic analyses indicated an increased TLS signature in *KDM6A*^{Mut} male patients, while the angiogenesis signature was predominantly enriched in *KDM6A*^{Mut} female patients (figure 4D). Similarly, in the ZSHS cohort, IHC assays disclosed a more prevalent presence of TLS in *KDM6A*^{Mut} male patients, especially when compared with *KDM6A*^{Mut} female patients (figure 4E, incidence rate: 35.1% vs 14.3%).

DISCUSSION

Analyses of UC have consistently highlighted an extraordinary prevalence of alterations in chromatin-modifying genes, a pattern distinctively more pronounced than in many other cancer types.[5 34] This underscores the pivotal role epigenetic deregulation might play in the pathogenesis of UC. Among the implicated genes, the X-linked *KDM6A* emerges as particularly gender-sensitive. It is noteworthy that gender disparities in UC continue to

be significant even when accounting for established risk factors such as smoking, occupational exposures and urinary tract infections. Our comprehensive investigation sought to decipher the complex relationship between gender and *KDM6A* mutations in UC. To this end, we leveraged seven diverse real-world datasets, which collectively offered a rich tapestry of clinical and genomic insights. This endeavour, to our knowledge, is the inaugural systematic exploration of the clinical ramifications of *KDM6A* mutations in UC across gender divides.

Apart from the MSKCC-IMPACT cohort—which noted mutation frequencies of 19.7% in men and 33.3% in women—other cohorts did not present significant gender-wise mutation frequency disparities. Remarkably, reduced KDM6A expression is associated with the progression of bladder cancer and indicates unfavourable disease-free survival in women; this pattern is not observed in their male counterparts.[3] In evaluating clinical outcomes of the patient with UC, our data suggest that those with *KDM6A* mutations marginally outlived their non-mutated peers. Factoring in gender, *KDM6A* mutations consistently conferred a survival advantage to men, while women with these mutations did not exhibit any survival differences associated with this mutation. Additionally, we observed that *KDM6A* mutations correlate with suppressed KDM6A mRNA expression, resonating with gender-specific findings from prior studies. Nonetheless, discerning if distinct thresholds for 'low' KDM6A expression are imperative across genders, especially given its XCI escape character, warrants deeper exploration.

Therapeutically, both chemotherapy and immunotherapy are linchpins in UC management. However, securing enduring disease control remains elusive for a substantial patient cohort. This has galvanised concerted efforts to pinpoint biomarkers that presage treatment response or resistance. Intriguingly, within the broader patient spectrum, those harbouring *KDM6A* mutations exhibited a propensity for enhanced OS under chemotherapy. For male recipients of chemotherapy, *KDM6A* mutations did not reveal any noteworthy clinical associations. Contrastingly, their female counterparts with *KDM6A* mutations not only showcased prolonged survival but also a notable absence of mortality events. Regarding immunotherapy, male patients with *KDM6A* mutations reported the most favourable outcomes, while their female counterparts with the same mutations experienced the least favourable results in terms of OS and response rate. This emphasises the dichotomous influence of *KDM6A* mutations on immunotherapy results contingent on gender. Contemporary studies have reiterated that gender disparities in cancer prognoses and tumour biology are not mere statistical artefacts—with men generally confronting more adversities than women.[35] Additionally, metastatic tendencies and therapeutic responses markedly vary between genders.[4] On amalgamating non-sex-specific cancers, male patients exhibited a higher occurrence of distant metastasis than women (pooled OR=1.06, 95% CI: 1.04 to 1.08; p<0.01). Additionally, male patients experienced poorer OS subsequent to distant metastasis (HR=1.08, 95% CI: 1.05 to 1.10; p<0.01).[36] Specifically concerning bladder cancer, global cancer analyses have underscored a stark disparity: men are four times more likely to develop bladder cancer than women.[37] Intriguingly, female gender was independently associated with a significantly higher risk of disease recurrence (HR=1.53; p=0.005).[38] This finding stands as a notable contrast to the general trend observed across most cancer types. Central to our conclusions is the clarion call for clinicians and researchers to factor in gender nuances at every juncture of medical decision making.

A profound grasp of the molecular disparities between male and female cancers is essential for tailored patient care and therapeutic strategies. The nuanced interactions of sex hormones and sex chromosomes critically influence aspects of oncogenesis, including the regulation of cancer-initiating cell populations, modulation of the tumour microenvironment and other systemic determinants such as immunological and metabolic factors.[39] Animal model research has postulated that while testosterone may potentiate bladder UC,[3] oestrogen seems to exert a protective role, although with the potential of accelerating growth in already established tumors.[40] Our research delved specifically into the ramifications of *KDM6A* mutations on sex hormone signalling pathways. Our analyses illuminated that, patients with *KDM6A* mutations manifested amplified AR activity while witnessing a decline in ESR1 activity, a trend consistent across gender divides. In breast cancer, the oestrogen receptor (ER) encoded by the ESR1 gene is pivotal. Hormonal therapy involves medications that either impede oestrogen action or reduce oestrogen levels. This intervention aims to modulate the activity of ESR1, thereby hindering the growth and proliferation of oestrogen-dependent cancer cells. In prostate cancer, persistent androgen-axis signalling mediated by adrenal, testicular and intratumoral androgen synthesis, and AR amplification and mutations in driving tumour growth have been increasingly acknowledged. The foundation of treatment of advanced prostate cancer is the suppression of gonadal androgens, which invariably leads to the development of castration-resistant disease. Several mechanisms have been identified to explain persistent androgen signalling in castration-resistant prostate cancer including increased AR gene expression and mutations in the AR gene.[41] Given the therapeutic deployment of hormone-modulating drugs in breast and prostate cancers, and the ongoing clinical endeavours assessing their relevance in UC, our revelations accentuate the necessity of weaving in gender-centric genomic insights into both future research and clinical paradigms.

Additionally, the evident and pronounced differences in response to ICIs advocate for integrating both immune and molecular characteristics.[42] According to the literature, a substantial majority of immune cells express receptors for sex hormones. A multitude of immune-centric genes present AR and ESR1 responsive elements within their promoter regions, positing that gender-differential immune responses could be a downstream effect of these molecular

influencers. The nuances of these responses hinge on a plethora of factors, including specific immune cell lineages, their anatomical positioning, hormonal flux and the spatial distribution of their receptors. Echoing this complexity, sex hormones have been documented to toggle between immune-stimulating and inhibitory roles, contingent on their concentrations and temporal dynamics.[43–45] In addition to the subtle interplay between hormone activity and the microenvironmental milieu, the genomic background, whether on the basis of neoantigen or the evaluation via SOPORANO, has also contributed to the differences in the clinical manifestations. Aligning with this perspective, our findings showcased that male patients with UC with *KDM6A* mutations harboured heightened densities of T cells, cytotoxic T cells and NK cells, likely steered by AR signalling. Furthermore, as *KDM6A* itself protects patients with UC from possible epigenetic dysfunctions in vivo,[3] its absence in female patients can therefore render their genomes fragile to cytotoxic therapies like cisplatin, which might account for the superior outcome for chemotherapy.[46] However, such defect in genome integrity, accompanied by the activities in hormone receptors, might also have simultaneously led to a different mutational spectrum and versatile survival benefit, leading to ultimately poor prognosis.[40 47 48] Nevertheless, the intricate interplay of sex hormones and immune dynamics within the context of UC remains an area ripe for deeper exploration.

Numerous studies have presented evidence that *KDM6A* can activate gene expression in a catalytic-independent manner. The absence of *KDM6A* leads to increased cell proliferation mediated by Enhancer of Zeste Homolog 2 (EZH2).[49] As the enzymatic catalytic subunit of polycomb repressive complex 2, EZH2 influences on downstream target genes by trimethylating Lys-27 in histone 3 (H3K27me3).[50] Dysregulation of EZH2, due to its impact on cell cycle progression, contributes to accelerated cellular proliferation, prolonged cell viability, and may play a role in the initiation and progression of cancer.[51 52] The crucial role of the FOXA1-KDM6A-ARHGDIB axis in restraining the malignancy and the efficacy of small-molecule inhibitors targeting EZH2 in combating KDM6A-null bladder cancer has been demonstrated in various mouse models,[49 53] advancing the development of these drug candidates. In addition, existing evidence shows that epigenetic therapy specifically targeting EZH2, either alone or in combination with cisplatin, holds promise for bladder tumours with *KDM6A* mutations by inducing NK cell-mediated differentiation and death.[54] Thus, EZH2 inhibition represents an additional therapeutic option that warrants comprehensive exploration in UC with *KDM6A* mutations.

In summation, our work emphasises the indispensability of recognising gender as a paramount determinant in decoding the complexities of oncological defenses. We advocate, based on both foundational and translational evidence, that infusing gender-centric genetic insights into oncological interventions holds the promise of refined therapeutic outcomes. The onus now is to recalibrate the trajectory of oncological medicine, ensuring bespoke and optimised treatment paradigms for both male and female patients.

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