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Research Article

RESEARCH ARTICLE

The interplay of cell cycle and DNA repair gene alterations in upper tract urothelial carcinoma: predictive and prognostic implications

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Abstract

Upper tract urothelial carcinoma (UTUC) is rare but can occur sporadically outside the context of Lynch syndrome. In these cases, knowing whether non-mismatch repair (MMR), DNA damage response and repair (DDR), and cell cycle gene alterations may predict responses to chemotherapy or immunotherapy and survival is of clinical importance. This study examined the germline and somatic mutational landscape of two UTUC patients with differential responses to programmed death 1 (PD-1)/PD-ligand 1 (PD-L1) immune checkpoint inhibitors and queried three independent UTUC cohort studies for co-occurrence of key cell cycle and DDR genes, as well as for their associations with overall survival (OS). TP53 and RB1 emerged as potential determinants of shorter OS in UTUC cohort patients, regardless of concurrent DDR alterations, and if prospectively assessed in larger studies they might also explain resistance to PD-1/PD-L1 blockade despite PD-L1 expression.

Key words: upper tract urothelial carcinoma; DNA damage response and repair; cell cycle; genetic testing; genomics; mutations

Introduction

Genomic aberrations in DNA damage response and repair (DDR) genes may predict favorable outcomes of patients with advanced urothelial carcinoma of the bladder (UCB), treated with platinum-based chemotherapy[1–5], or immune checkpoint blockade targeting programmed death 1 (PD-1) or its ligand (PD-L1).[6] Additionally, some DDR genes (e.g. ATM, ATR) are cell cycle checkpoints that mediate downstream signals or/and directly interact with key cell cycle regulators such as p53 and RB1.[7] A more complex prognostic role has been suggested in multicenter retrospective cohorts whereby ATM alterations were associated with a shorter overall survival (OS).[8]

Upper tract urothelial carcinoma (UTUC) is only a minority of UC (5%–10%) and is frequently associated

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Table 1. Demographics and clinicopathological characteristics of two UTUC patients with differential response to PD-1/PD-L1 checkpoint blockade.

Characteristics	Patient 1	Patient 2
Age at diagnosis	87	92
Gender	Male	Female
Race/ethnicity	African American	White, Jewish Ashkenazi
Smoking status	Never smoker	Never smoker
Personal history of cancer	Bladder (urothelial)	Skin (melanoma, squamous cell carcinoma)
Family history of cancer	Niece—breast	Father—skin (SCC) Sister—bladder (UC) Son—skin (melanoma, SCC)
Tumor location	Renal pelvis	Ureter
Clinical stage	T3N0M0	T4N0M0
Grade	high	high
Prior chemotherapy	No	No
Prior surgery	No	No
Best response to PD-1/PD-L1 blockade	PR	PD
Progression-free survival (mos from diagnosis)	NR (8 to present)	4
Time to response (mos)	2	NA
Duration of response (mos)	NR (8 to present)	NA
PD-L1 IHC expression (%)	100	10

Abbreviations: IHC, immunohistochemical; mos, months; NA, not applicable; NR, not reached; PD, Progression of disease; PD-L1, programmed death ligand 1; PR, partial response; SCC, squamous cell carcinoma; UC, urothelial carcinoma.

with germline defects of mismatch repair (MMR) genes causing Lynch syndrome.[9,10] Because UTUC is often underrepresented in UC studies, the impact of DDR alterations outside the spectrum of MMR genes on clinical outcomes is less well studied. Additionally, whether alterations of cell cycle genes may be clinically relevant in the outcomes of patients with DDR-deficient UTUC is not fully understood in UTUC.

This study examined the germline and somatic mutational landscape of two UTUC patients with differential response to programmed death 1 (PD-1)/PD-ligand 1 (PD-L1) immune checkpoint inhibitors and queried three independent UTUC cohort studies for co-occurrence of key cell cycle and DDR genes as well as for their associations with OS.

Materials and methods

Two patients with UTUC of right renal pelvis (patient 1) and left ureter (patient 2), respectively, with differential responses to frontline therapy with PD-1/PD-L1 inhibition were studied. The clinical, pathological characteristics, and response to therapy were captured.

To investigate the underlying biology of the patients' differential responses, germline and somatic sequencing analyses from these two inoperable, platinum-ineligible patients were conducted. Tumor tissue for patient 1 was obtained via CT-guided percutaneous biopsy of the right kidney. Tumor tissue for patient 2 was obtained via left ureteroscopy and ureteral biopsy.

Tumor specimens and peripheral blood were subjected to next-generation sequencing analysis using (i) a 324-gene panel for somatic testing and (ii) an 87-gene panel consisting of known cancer genes that may be involved in different hereditary cancer syndromes, as previously described by the manufacturers, respectively.[11,12] In patient 2, both tumor sequencing and circulating tumor DNA (ctDNA) sequencing were performed.

Publicly available datasets of 251 UTUC tumor samples from 249 patients were queried for frequency of DDR and cell cycle gene alterations as well as their associations with OS.[13–15] All statistical analyses were conducted on cBioportal for cancer genomics (www.cbioportal.org) and 0.05 was set as a cut-off for statistical significance of *P*- and *q*-values.

Results

The clinical and pathological characteristics of the patients are presented in Table 1. Patient 1 (responder) demonstrated partial response (PR) on two consecutive radiographic assessments, at 2 and 5 months after initiation of atezolizumab, and remains on PD-L1 blockade (Fig. 1), whereas patient 2 (progressor) experienced progression 4 months after initiation of pembrolizumab (Fig. 2). Tumors from both patients tested positive for immunohistochemical (IHC) expression of PD-L1 (responder 100%, progressor 10%). The patients' tumors (tumor-only in patient 1, tumor and liquid biopsy in patient 2) harbored pathogenic mutations in the DDR gene, ATM, as well as additional alterations in different DDR or/and cell cycle genes (responder, PALB2-somatic and CDKN1A-somatic; progressor, RB1-germline and TP53-somatic) (Table 2).

It was hypothesized that cell cycle gene aberrations, particularly those co-occurring with DDR alterations found in these two patients, might play a role in different outcomes observed in these two patients. To address

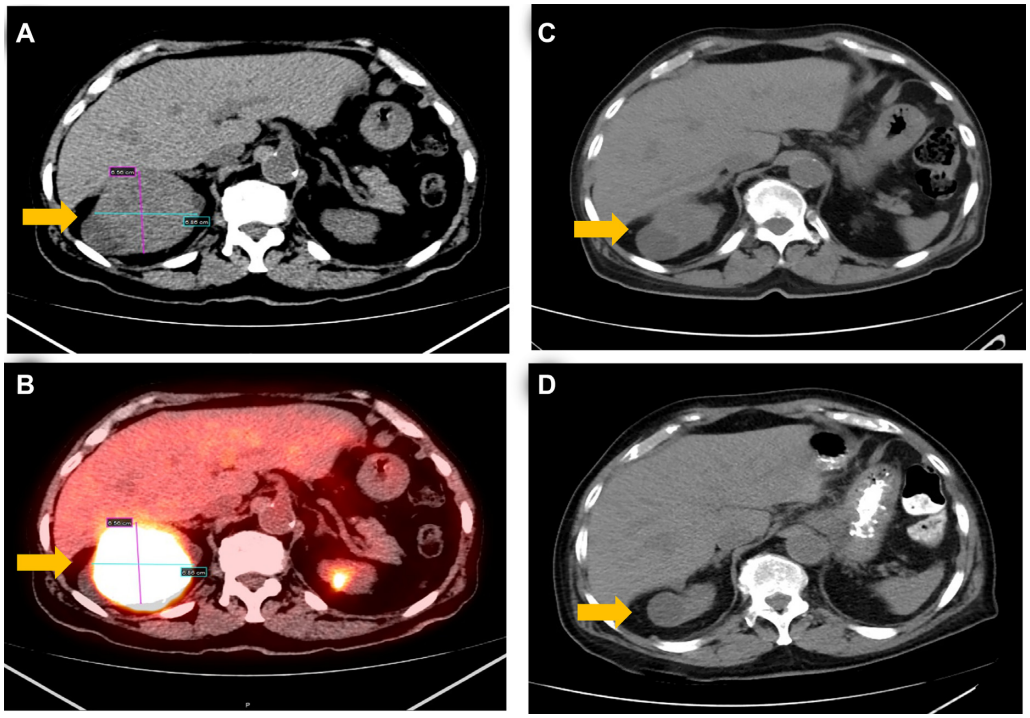

Figure 1. Patient 1: CT (**A**) and PET scan (**B**) showing a large hypermetabolic mass in the upper pole the right kidney, measuring 7.2 cm × 6.6 cm × 7 cm, with standard uptake values (SUV) 17.3–25.5. Repeat CTs of the abdomen and pelvis after 2 (**C**) and 5 months of immunotherapy (**D**), respectively, showing continuous interval decrease in size of the mass.

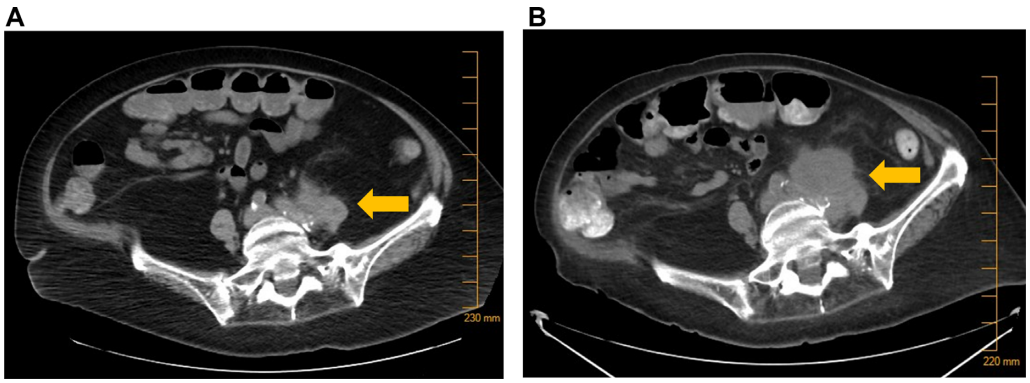

Figure 2. Patient 2: (**A**) CT scan of the abdomen pelvis showing a mass involving the left ureter, inseparable from the medial surface of the left psoas muscle and the anterolateral surface of the proximal portion of the left common iliac artery measuring 3.7 cm × 3.3 cm, associated with infiltrative changes within the adjacent portion of the retroperitoneal fat as well as thickening of the left posterior peritoneal fascia. (**B**) Repeat CT 4 months later showing significant interval enlargement of the mass now measuring 4.0 cm × 4.5 cm.

this hypothesis, DDR and cell cycle genes were queried in publicly available datasets of 251 UTUC tumor samples from 249 patients for their frequencies, co-occurrence, and associations with OS.[13–15] The frequency of ATM alterations in three previously described UTUC cohorts was 15%, the majority of which included missense mutations ($n = 48$), followed by nine truncating and two non-start mutations (Fig. 3A). PALB2 gene was altered in only 3% of these UTUC samples/patients (eight missense mutations) (Fig. 3B). The overall frequency of alterations within a group of 34 well-described DDR genes[5,6] in UTUC in these three cohorts was as high as 32% (78/241). The presence of somatic alterations in any of 34 DDR genes was not associated with OS (log-rank $P = 0.309$) (Fig. 3C). Intriguingly, when Kaplan Meier analysis was repeated after excluding ATM alterations, there was a trend toward improved OS in these patients (log-rank $P = 0.086$) (Fig. 3D). These findings are in concordance with a previously reported negative prognostic role of ATM somatic mutations in relapsed/advanced UC of predominantly bladder primary.[3]

Analysis of cell cycle regulatory genes in all three UTUC cohorts revealed that the most frequently altered was TP53 (22%) displaying 43 missense and 20 truncating mutations, followed by CDKN2A (15%) and CDKN2B (14%) (Fig. 4A). CDKN1A was found to be mutated in 8% of cases (Fig. 4B) and RB1 mutations were less common (2.1%) (Fig. 4C).

Table 2. Germline and somatic alterations (pathogenic) in two UTUC patients with differential response to PD-1/PD-L1 checkpoint blockade.

	Molecular alterations			
	Patient 1 (responder)		Patient 2 (progressor)	
Gene (DDR, cell cycle)	Germline	Somatic	Germline	Somatic
ATM		2922–2A > T	c.663–3C > G (Intronic) VUS	splice site 3077 + 1G > A
			c.8762C > A (p.Thr2921Lys)	splice site 6198 + 1G > A
			VUS	Y2954C
PALB2		R879*		
BRCA2	c.2274T > G (p.Ser758Arg) VUS			
TP53				N310fs*35
CDKN1A		R94fs*35		
RB1			c.1399C > T (p.Arg467*) pathogenic	R467
Gene (other)				
FGFR3		Y373C amplification		
NOTCH3		G2149fs*102		
IDH1		R132C		
TSC1		splice site 364–2A > T		
CD70		C151*		
DNMT3A		R882H		
TERT		promoter -124C > T		
TET2		K203*		
MLL2				R3707*

Abbreviations: VUS, variant of uncertain significance.

It was hypothesized that TP53, RB1, or CDKN1A mutations seen in patients 1 and 2, both of whom also harbored somatic ATM mutations, could exert a potential effect on their differential outcomes. To test this hypothesis, these genes were queried independently and together in the three combined UTUC datasets for their association with OS. Presence of ATM alterations alone did not appear to significantly affect OS in UTUC (log-rank $P = 0.33$) (Fig. 5A). This finding did not change when ATM was queried in combination with PALB2 and CDKN1A (log-rank $P = 0.778$), a mutation co-occurrence seen in patient 1 (responder) (Fig. 5B). Importantly, alterations in ATM and PALB2 genes had a statistically significant co-occurrence in UTUC ($q < 0.001$) and same was the case for the co-occurrence of PALB2 and CDKN1A ($q = 0.027$). When ATM was queried in combination with the cell cycle tumor suppressor genes RB1 and TP53 (a mutation co-occurrence seen in patient 2), OS was significantly shorter compared to patients without these gene alterations (log-rank $P = 9.459e\text{-}3$) (Fig. 5C). This outcome might be particularly "driven" by TP53 and RB1 since the presence of these mutations was associated with worse prognosis (Fig. 5D). Alterations in all three genes can significantly co-occur in UTUC (ATM/RB1 $q < 0.001$; ATM/TP53 $q = 0.026$; RB1/TP53 $q = 0.013$).

Within the three independent UTUC cohorts, a proportion of patients received neoadjuvant or adjuvant platinum-based chemotherapy (Supplementary Fig. 1). It is possible that this may have affected the outcomes of these patients. However, the use of chemotherapy in the neoadjuvant or adjuvant setting was balanced between the altered and unaltered groups in the entire meta-dataset including all 251 samples from 249 patients, according to: (i) DDR status (DDR including ATM: neoadjuvant $q = 0.843$; adjuvant $q = 0.843$; DDR excluding ATM: neoadjuvant $q = 0.757$; adjuvant $q = 0.757$), (ii) ATM status (neoadjuvant $q = 0.628$; adjuvant $q = 0.922$), (iii) ATM/PALB2/CDKN1A status (neoadjuvant $q = 0.970$; adjuvant $q = 0.970$), (iv) ATM/RB1/TP53 status (neoadjuvant $q = 0.753$; adjuvant $q = 0.264$), and (v) TP53/RB1 status (neoadjuvant $q = 0.499$). There was a trend toward more frequent use of adjuvant chemotherapy in the group of patients/tumors harboring TP53/RB1 alterations (9/14, 64.3%) compared to those unaltered (19/68, 27.9%), $q = 0.071$ (Supplementary Fig. 1).

Discussion

This study examined the potential role of germline or/and somatic cell cycle and DDR aberrations in shaping the clinical outcomes of patients with advanced UTUC. The study findings suggest that mutations in cell cycle regulatory genes, particularly TP53 and RB1, might modulate the predictive and prognostic impact of several DDR

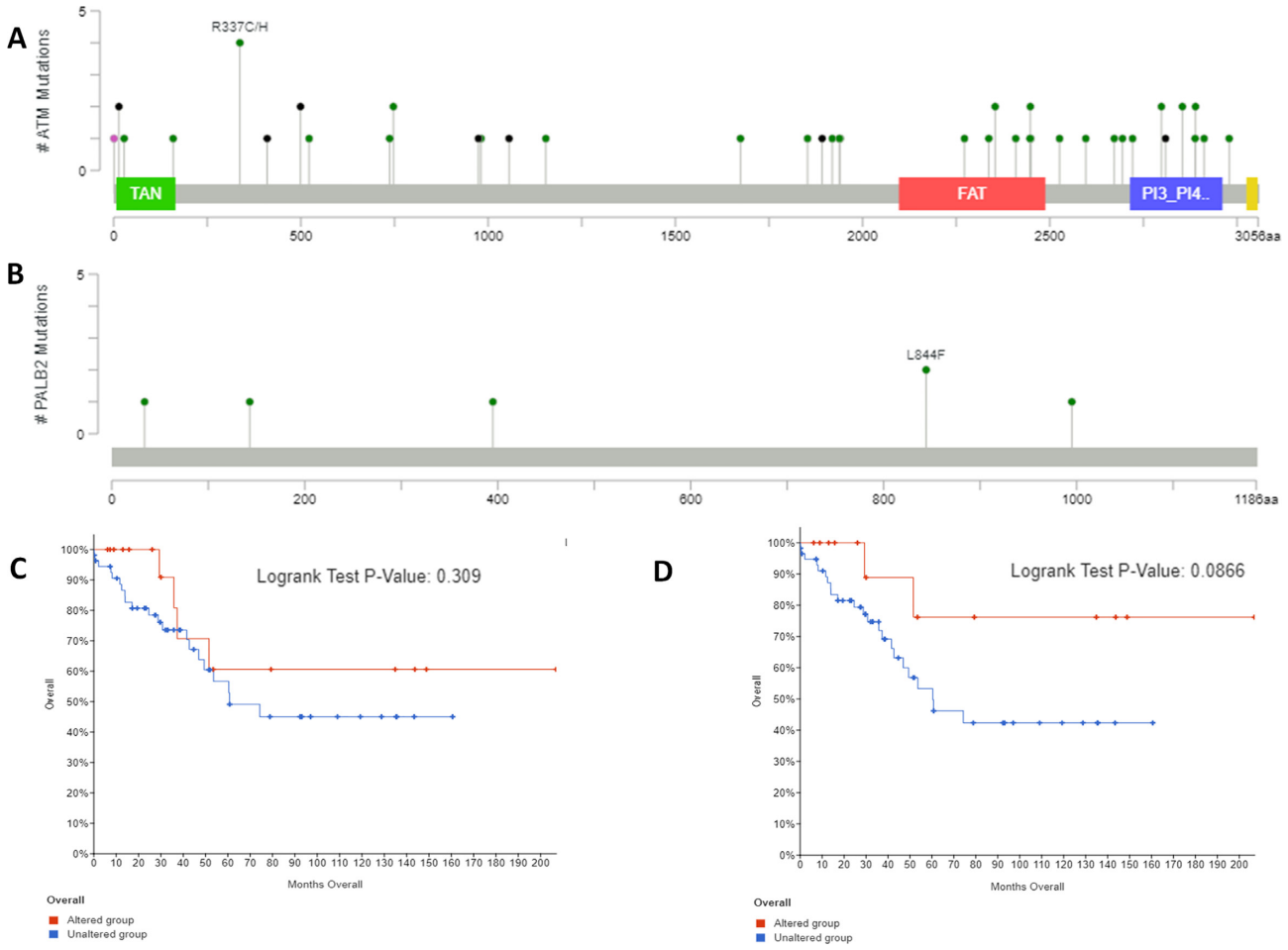

Figure 3. Lollipop plots of (**A**) ATM and (**B**) PALB2 gene somatic alterations across three UTUC cohorts. Missense mutations are indicated in green while truncating mutations are marked in black. Purple represents nonstart mutations. (**C**) Kaplan Meier curve of UTUC patients with/without DDR somatic alterations. (**D**) Kaplan Meier curve of UTUC patients with/without DDR somatic alterations, excluding ATM.

gene alterations on responses to PD-1/PD-L1 checkpoint inhibition or/and survival.

With respect to the potential etiology for the occurrence of UTUC in the two subjects with detailed clinical and molecular characteristics presented, patient 1 had no pathogenic germline mutations and a personal history of UCB, suggesting sporadic disease. Indeed, representative studies and meta-analyses showed that, further to an increased risk of 1%–5% of metachronous UTUC in patients with UCB, there is a possible clonal relatedness in up to 85% of cases.[13,16] In contrast, patient 2 was found to have a germline RB1 mutation that was likely involved in the pathogenesis of her UTUC and prior skin melanoma. Although somatic mutations of RB1 are less frequent in UTUC compared to UCB,[13–15] a significantly elevated risk of UC has been reported in germline RB1 mutation carriers and retinoblastoma survivors after >30 years of follow-up[17]. In a recent analysis of the prevalence of pathogenic cancer risk variants in patients with high-risk UC, two out of 303 patients tested for RB1 (0.7%), within a larger cohort of 1038 patients, were found to have pathogenic germline mutations in the RB1 gene.[18,19] BRCA2 and ATM heritable mutations have also been associated with an increased risk of UC;[18–20] however, patient 1 and patient 2 in this study were carriers of variants of uncertain significance with respect to these two genes.

The role of differential PD-L1 expression between patient 1 (responder 100%) and patient 2 (progressor 10%) in the different clinical outcomes of these two patients is not entirely clear; however, it is possible that the higher PD-L1 expression in patient 1 might have been associated with a higher likelihood of response to PD-L1 inhibition. Most evidences on the predictive or/and prognostic significance of PD-L1 emanate from UCB patients (not UTUC) progressing after chemotherapy, and are limited by discordance in different assay methodologies and clinical trial designs.[21] For example, in the KEYNOTE-045 phase III randomized study, PD-L1 expression on both tumor cells (TC) and immune cells (IC), known as combined positive score (CPS) ≥10 correlated with worse OS whereas in the Imvigor211 phase III randomized trial, IC PD-L1 ≥5% was predictive of improved objective response rate.[22–24] In meta-analyses including UC among other tumor types, higher TC PD-L1 expression has been associated with a worse prognosis.[25] In the first-line setting in cisplatin-ineligible patients, the phase II Imvigor 210 study reported responses in all IC PD-L1 expression

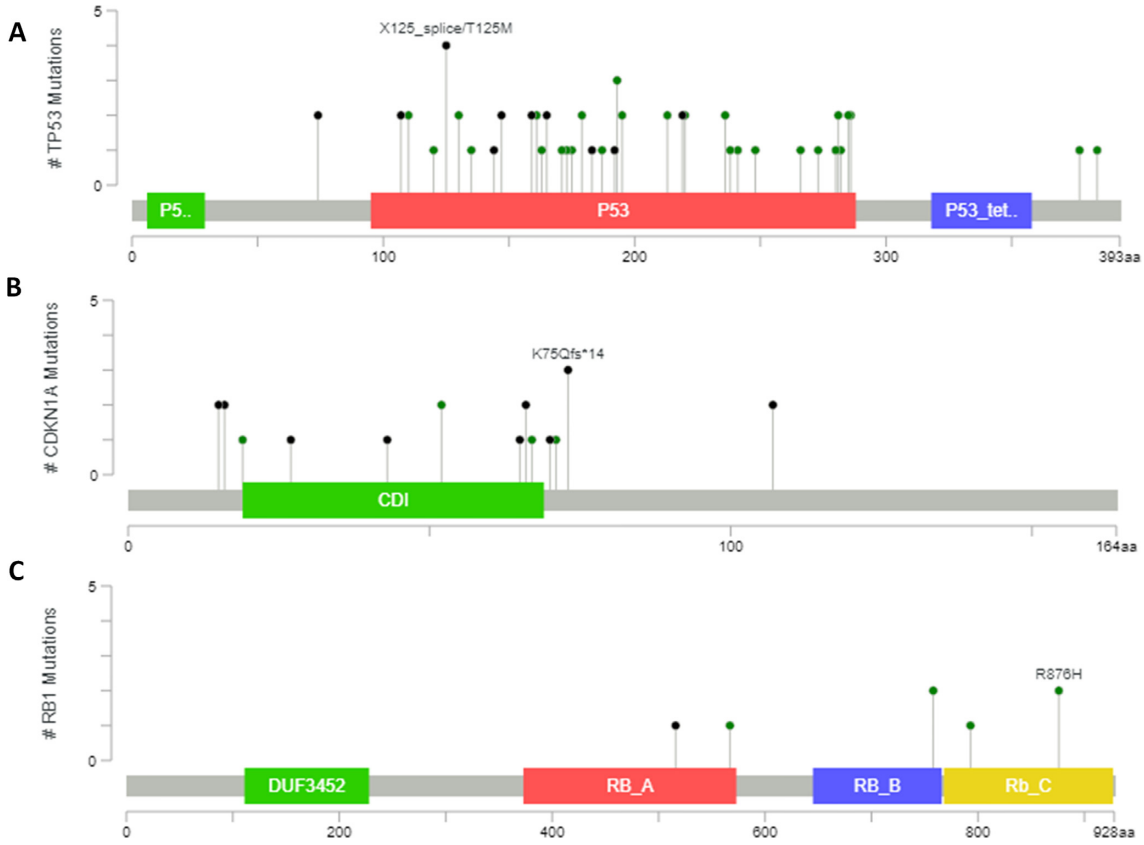

Figure 4. Lollipop plots of (**A**) TP53, (**B**) CDKN1A, and (**C**) RB1 gene somatic alterations across three UTUC cohorts. Missense mutations are indicated in green while truncating mutations are marked in black.

groups.[26] Median OS was shorter in patients with IC PD-L1 expression ≥5%, compared to those with <5% (12.3 months versus 19.1 months, respectively) and unlike in the second-line setting, there was no statistically significant difference in response depending on PD-L1 status.[26] In the KEYNOTE-052 phase II study, although a CPS of at least 10% enriched for response to first-line pembrolizumab (38%), low or absent PD-L1 expression did not preclude responses.[27] Interestingly, in retrospective cohorts of UTUC-only patients, cancer-specific survival was not significantly associated with positive PD-L1 expression using a 5% cut-off in one study; whereas in another study PD-L1 positivity ≥1% of tumor cells was predictive of favorable OS only in organ-confined disease, as opposed to PD-1 positivity ≥1% of tumor infiltrating lymphocytes that emerged as an independent prognosticator of worse cancer-specific survival and OS.[28,29] Efforts have been made by other groups to explain these discordances by accounting for additional clinical factors, such as platelet (PLT) count that may "interact" with PD-L1 and result in strengthening (in patients with high PLT counts) or weakening (in patients with low PLT counts) the prognostic impact of PD-L1.[30] Additionally, whether there is a "gradient" of PD-L1 expression that correlates with response or/and OS remains poorly understood as existing studies have assessed cut-off values and did not compare different levels of PD-L1 positivity with each other.

The results of this analysis of cell cycle and DDR genes from publicly available datasets of 251 UTUC tumor samples from 249 patients on their frequencies, co-occurrence, and associations with OS conducted by this study are in line with prior observations that ATM/RB1 mutations are predictive of shorter OS in unselected UC patients.[31] Although TP53 and RB1 mutations are less frequent in UTUC compared to UCB,[13–15] p53 expression (as a result of TP53 mutations) has been associated with advanced stage, high-grade disease, and female gender.[32] Additionally, the absence of functional RB1 after germline (patient 2) or somatic deleterious alterations is linked to immune evasion.[33]

This study was limited by retrospective nature as well as a potential lack of capturing the full spectrum of tumor heterogeneity in the biopsy specimens obtained (particularly for patient 1), as opposed to examination of the entire primary tumor. Additionally, some patients included in the three UTUC cohorts underwent neoadjuvant or adjuvant chemotherapy, which may have affected outcomes.

In conclusion, the results of this hypothesis-generating analysis suggest that defects in cell cycle regulatory genes, particularly TP53 and RB1, might

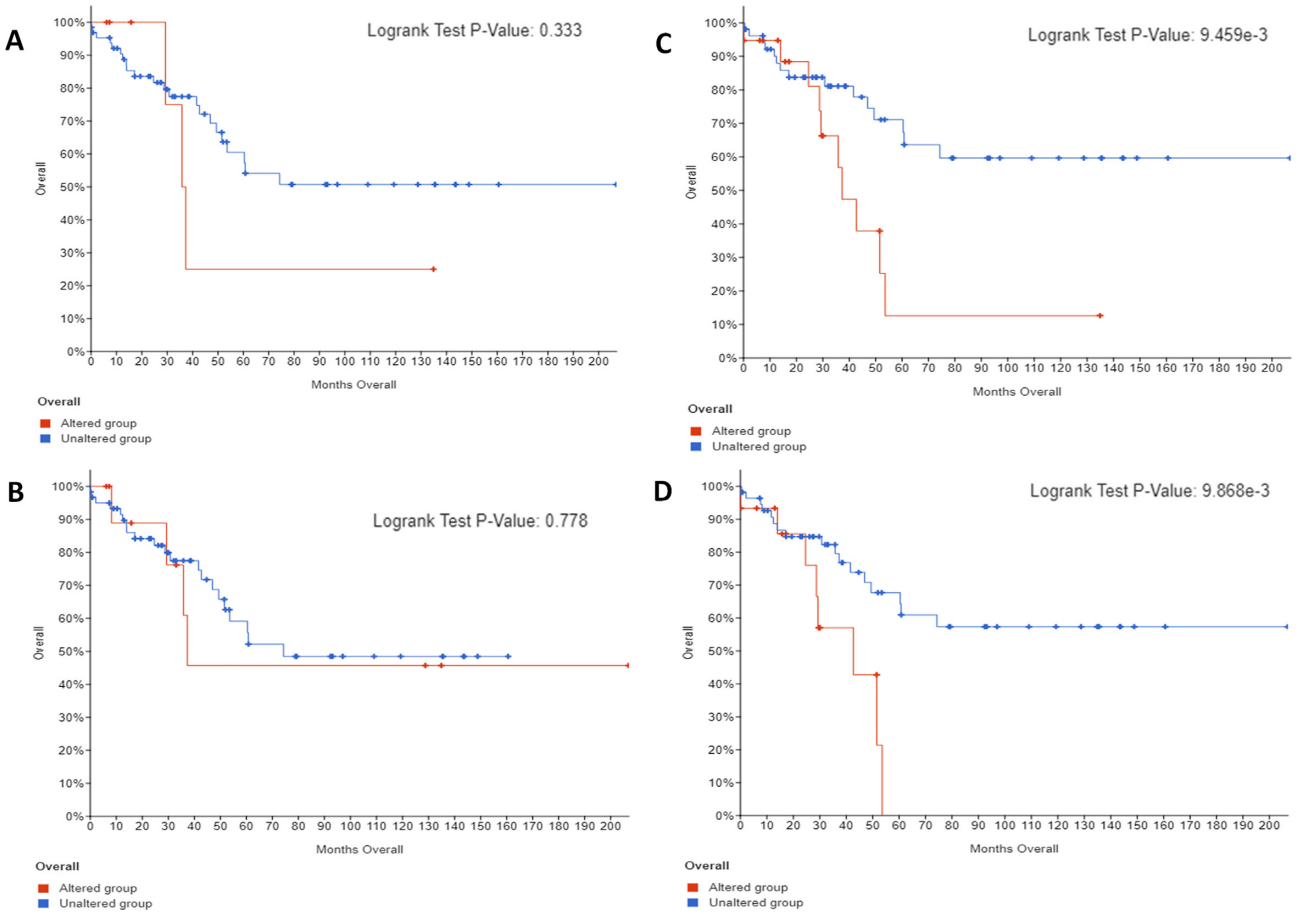

Figure 5. Kaplan Meier curves of UTUC patients (**A**) with/without ATM somatic alterations, (B) with/without ATM, PALB2, CDKN1A somatic alterations, (**C**) with/without ATM/RB1/TP53 somatic alterations, and (**D**) with/without TP53/RB1 somatic alterations.

modulate the predictive and prognostic impact of several DDR gene alterations on responses to PD-1/PD-L1 checkpoint inhibition or/and survival, thus they should be accounted for in future prospective correlative biomarker studies in UC.

Supplementary material

Supplementary data is available online at *PCMEDI*.

Conflict of interest

None declared.

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