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TERT promoter mutations and other prognostic factors in patients with advanced urothelial carcinoma treated with an immune checkpoint inhibitor

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ABSTRACT

Background Immune checkpoint inhibitors (ICI) can achieve durable responses in a subset of patients with locally advanced or metastatic urothelial carcinoma (aUC). The use of tumor genomic profiling in clinical practice may help suggest biomarkers to identify patients most likely to benefit from ICI.

Methods We undertook a retrospective analysis of patients treated with an ICI for aUC at a large academic medical center. Patient clinical and histopathological variables were collected. Responses to treatment were assessed for all patients with at least one post-baseline scan or clear evidence of clinical progression following treatment start. Genomic profiling information was also collected for patients when available. Associations between patient clinical/genomic characteristics and objective response were assessed by logistic regression; associations between the characteristics and progression-free survival (PFS) and overall survival (OS) were examined by Cox regression. Multivariable analyses were performed to identify independent prognostic factors.

Results We identified 119 aUC patients treated with an ICI from December 2014 to January 2020. Genomic profiling was available for 78 patients. Overall response rate to ICI was 29%, and median OS (mOS) was 13.4 months. Favorable performance status at the start of therapy was associated with improved OS (HR 0.46, p=0.025) after accounting for other covariates. Similarly, the presence of a *TERT* promoter mutation was an independent predictor of improved PFS (HR 0.38, p=0.012) and OS (HR 0.32, p=0.037) among patients who had genomic profiling available. Patients with both a favorable performance status and a *TERT* promoter mutation had a particularly good prognosis with mOS of 21.1 months as compared with 7.5 months in all other patients (p=0.03).

Conclusions The presence of a *TERT* promoter mutation was an independent predictor of improved OS in a cohort of aUC patients treated with an ICI who had genomic data available. Most of the clinical and laboratory variables previously shown to be prognostic in aUC patients treated with chemotherapy did not have prognostic value among patients treated with an ICI. Genomic profiling may provide important prognostic information and affect clinical decision making in this patient population. Validation of these findings in prospective patient cohorts is needed.

BACKGROUND

Immune checkpoint inhibitors (ICI) have revolutionized the management of metastatic and locally advanced urothelial carcinoma of the bladder and urinary tract (aUC). Starting with atezolizumab in May of 2016, five ICIs are now approved for the treatment of aUC after progression on a platinum-based chemotherapy regimen.[1–5] Cisplatin-ineligible patients may also receive pembrolizumab or atezolizumab in the front-line setting,[6–8] while avelumab was recently granted Food and Drug Administration (FDA) approval as switch maintenance therapy following first-line platinum-based chemotherapy.[9] Yet while ICIs can achieve durable responses in a subset of patients, only 20%–25% of patients respond to immunotherapy[1–8] and reliable predictors of response to ICI are lacking. As alternative targeted therapies emerge for patients with aUC,[10 11] there is an urgent need for novel biomarkers to help identify patients most likely to benefit from ICI treatment.

Existing data on the predictive value of programmed death-ligand 1 (PD-L1) expression in aUC are mixed: high PD-L1 expression was associated with response to post-platinum atezolizumab in the initial phase 2 IMvigor 210 study and to front-ine pembrolizumab in the KEYNOTE-052 study,[7 12] however, durable responses can still be seen in patients with low PD-L1 expression.[13] Conversely, low PD-L1 expression may predict inferior outcomes in patients receiving front-line ICI.[8 14] Tumor mutation burden (TMB) is thought to

contribute to tumor immunogenicity through increased neoantigen expression, and in June 2020 pembrolizumab was granted accelerated FDA approval for the treatment of advanced solid tumors with a high TMB that have progressed on prior therapy.[15] Retrospective evidence in aUC suggests that a high TMB may indeed predict clinical benefit to ICI,[16 17] and a multivariable analysis of clinical and genomic factors in aUC showed that high TMB—along with low neutrophil to lymphocyte ratio (NLR) and lack of visceral metastases—was associated with response to immunotherapy.[18] A prespecified subgroup analysis of IMvigor130 failed to demonstrate a survival benefit of front-line atezolizumab (alone or in combination with chemotherapy) over platinum-based chemotherapy in patients with a high TMB, although a smaller subset of patients who had both high PD-L1 expression and high TMB did seem to derive more benefit from atezolizumab relative to platinum-based chemotherapy.[19] Prospective validation of TMB as a biomarker of response to ICI in aUC is still needed.

Genomic profiling using next-generation sequencing (NGS) is increasingly used in the management of cancer patients and patients with aUC. We hypothesized that the use of this real-world genomic data—in combination with baseline clinical and laboratory features—may help to identify novel independent predictive markers of response to immunotherapy. Here, we present the results of a single-center retrospective analysis of the clinical and genomic factors associated with clinical outcomes among aUC patients treated with an ICI.

METHODS

Patient and data collection

Patients treated with ICI monotherapy for aUC at the University of California, San Francisco from December 2014 to January 2020 were included in this retrospective analysis. Patient data were collected from electronic medical record review in compliance with institutional review board guidelines. Patient eligibility criteria included: histologically confirmed UC, presence of locally advanced or metastatic disease, at least one dose of an ICI administered (including atezolizumab, pembrolizumab, nivolumab, durvalumab or avelumab), and available clinical, pathologic and imaging data prior to initiation of treatment. To be considered eligible for response assessment, a patient needed to have at least one scan following initiation of an ICI or clear evidence of clinical progression as assessed by the treating physician. Patients who received an ICI for an indication other than aUC were excluded from this analysis.

Baseline clinical and laboratory characteristics were collected for each patient. Results of testing for PD-L1 expression via the PD-L1 IHC 22C3 pharmDx assay (NeoGenomics Laboratories) were also collected when performed at the discretion of the treating clinician. Tumor genomic profiling was performed using Clinical Laboratory Improvement Amendments (CLIA) certified commercially available (FoundationOne and StrataNGS) NGS platforms, or a CLIA certified institutional NGS assay (UCSF 500 Cancer Gene Panel Test, which uses hybrid capture enrichment of target DNA to interrogate 479 common cancer genes). For some patients, pathogenic germline mutations were also identified on commercially available CLIA-certified NGS platforms (Ambry Genetics, Myriad Genetics and Invitae), and thus were also included in this analysis.

Assessment of objective response (defined as a complete response (CR) or partial response (PR)) or progression was determined based on the judgment of the investigator assessing the patient's chart using the available information from radiographic reports or clinical notes. Response assessment in patients who received >1 ICI in sequence was performed only during the first course of ICI therapy. Duration of response was defined as the time from the first documented clinical or radiographic response to progression, death or time of last follow-up for patients who had not yet progressed on ICI. Progression-free survival (PFS) was defined as the time from ICI start to progression or death; patients alive without disease progression at last follow-up were censored at the date of last follow-up. Overall survival (OS) was defined as the time from ICI start until death; those alive at last follow-up were censored at the date of last follow-up. PFS and OS in patients who received >1 ICI in sequence were defined from the start of the first course of ICI therapy and objective response rate (ORR) was assessed only with the first ICI therapy.

Statistical analysis

Summary statistics were used to describe baseline patient and treatment characteristics, as well as PD-L1 expression status, TMB and genomic alterations identified by NGS when available. Wilcoxon rank-sum test was used to compare TMB between patients with or without specific genomic alterations. Univariable analysis was performed to assess for correlations between clinical outcomes (response, PFS and OS) and[1] the top 20 most commonly altered genes,[2] relevant baseline demographic and clinical characteristics (age, location of primary tumor, histology, Eastern Cooperative Oncology Group (ECOG) performance status score, front-line versus postplatinum treatment setting, presence of visceral metastases, body mass index and[3] laboratory variables (albumin, hemoglobin, creatinine levels and NLR). Logistic model was used for binary response outcome and Cox proportional hazard (cph) model was used for time-to-event outcomes (ie, PFS and OS). To account for possible confounders and assess the independent effect of specific variables on treatment outcomes, multivariable logistic regression and Cox proportional hazard models were applied.

Four prespecified prognostic variables (albumin and hemoglobin levels, ECOG score, and the presence of visceral metastases) were selected a priori for the multivariable analyses, based on the existing literature.[20–22]

Additional variables with p<0.1 in the corresponding univariable analysis were included in the multivariable analyses. Two separate analyses for ORR, PFS and OS were performed[1]: using only clinical variables in the entire patient cohort and[2] using combined clinical and genomic variables in the subset of patients who had undergone genomic profiling. Statistical significance was set at a p<0.05. Adjustment for multiple testing was not performed. All analyses were conducted using the R statistical computing software (http://www.r-project.org).

RESULTS

Baseline patient characteristics

We identified 119 patients treated with an ICI for aUC from December 2014 to January 2020. Primary site of disease was bladder for 90 patients (75.6%), or upper genitourinary tract for 29 (24.4%) of patients. Sixty-three of 119 patients (52.9%) had undergone prior definitive surgery, and 50 (79.4%) of those patients had received neoadjuvant chemotherapy. The most common histological pattern was pure UC in 77 (64.7%) patients, while 14 (11.8%) patients had pure variant histology or variant-predominant histology (squamous cell carcinoma in 7, neuroendocrine in 2, adenocarcinoma in 2, other histology in 3). The remaining 28 patients (23.5%) had mixed histology that was urothelial predominant (with a component of squamous cell, plasmacytoid or micropapillary histology in 17, 4 and 4 cases, respectively; other histology in the remaining 3 cases). With regards to ICI treatment, the majority of patients received pembrolizumab (68.1%) or atezolizumab (29.4%), with the remainder receiving nivolumab (1.7%) and durvalumab (0.8%). Roughly half of all ICIs were administered in the postplatinum metastatic setting (61 out of 119 patients, 51.3%), with the remaining 58 patients (48.7%) receiving ICI in the front-line or treatment-naive metastatic setting; six patients received more than one ICI in sequence. A total of 78 of the 119 (65.6%) patients had genomic profiling data available. Other baseline characteristics at the start of ICI therapy are summarized in table 1.

Table 1 Baseline characteristics at the start of immune checkpoint inhibitor therapy

Characteristics	Entire cohort (n=119)	Patients with available genomic data (n=78)
Age, years—median (IQR)	71 (65, 77)	71 (66, 76)
Male—n (%)	77 (64.7)	49 (62.8)
Female—n (%)	42 (35.3)	29 (37.2)
Smoking history (present or former)—n (%)	71 (59.7)	46 (59.0)
Ethnicity—n (%)		
White	81 (68.1)	54 (69.2)
Asian	19 (16.0)	12 (15.4)
African American	6 (5.0)	5 (6.4)
Hispanic	4 (3.4)	2 (2.6)
Other	7 (5.9)	4 (5.1)
Primary bladder tumor—n (%)	90 (75.6)	57 (73.1)
Upper tract disease—n (%)	29 (24.4)	21 (26.9)
Cystectomy or nephroureterectomy—n (%)	63 (52.9)	47 (60.3)
Histology—n (%)		
Pure urothelial histology	77 (64.7)	47 (60.3)
Mixed variant histology	36 (30.3)	27 (34.6)
Pure variant histology	6 (5.0)	4 (5.1)
Immunotherapy treatment setting—n (%)		
Front-line metastatic	58 (48.7)	37 (47.4)
Postplatinum	61 (51.3)	41 (52.6)
ECOG PS—n (%)		
0–1	66 (55.5)	49 (62.8)
≥2	25 (21.0)	11 (14.1)
Unknown	28 (23.5)	18 (23.1)
Visceral metastases—n (%)	90 (75.6)	55 (70.5)
BMI, kg/m^2—median (IQR)	24.9 (22.0, 28.6)	25.2 (22.0, 28.8)
Hemoglobin <100 g/L—n (%)	37 (31.1)	26 (33.3)
Creatinine, mg/dL—median (IQR)	1.35 (1.03, 1.75)	1.41 (1.09, 1.78)
Albumin, g/dL—median (IQR)	3.4 (3.0, 3.8)	3.6 (3.0, 3.8)
NLR <5—n (%)	65 (54.6)	46 (59.0)

BMI, body mass index; NLR, neutrophil to lymphocyte ratio; ECOG PS, Eastern Cooperative Oncology Group performance status.

Treatment outcomes

Among all 119 patients treated with ICI, ORR was 29%, including 13% (n=16) CR and 15% (n=18) PR. An additional 16 patients (13%) achieved stable disease (SD) as their best response, for a disease control rate (DCR) of 42%. With a median follow-up of 6.3 months in this study, median PFS was 2.6 months (95% CI 2.01 to 4.34 months) and median OS was 13.4 months (95% CI 11.3 to 20.7). Among patients who achieved CR or PR, the median duration of response was 13.4 months (IQR 4.7–22.5). Relative to patients with mixed or pure variant histology (n=42), patients with pure urothelial histology (n=77) had more favorable PFS (median PFS 3.36 vs 1.88 months, p=0.04) and a trend towards more favorable OS (median OS 15.5 vs 12.3 months, p=0.07). There were no statistically significant differences in clinical outcomes among patients with primary bladder and primary upper tract tumors or between patients receiving ICI in the front-line versus postplatinum setting.

Table 2 Univariable analysis of objective response, progression-free survival and overall survival with relevant clinical and genomic characteristics

	Objective response		Progression free Survival		Overall survival	
Characteristics	**OR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Age	1.00 (1.00 to 1.01)	0.28	0.99 (0.97 to 1.01)	0.20	1.00 (0.98 to 1.03)	0.90
Bladder versus upper tract UC	0.89 (0.73 to 1.08)	0.23	1.03 (0.64 to 1.66)	0.91	1.47 (0.80 to 2.70)	0.22
Histology (pure UC vs mixed or pure variant histology)	1.17 (0.99 to 1.39)	0.08	0.64 (0.42 to 0.99)	**0.04**	0.63 (0.37 to 1.05)	0.08
Front line versus postplatinum	1.09 (0.92 to 1.28)	0.33	0.84 (0.55 to 1.27)	0.40	1.12 (0.68 to 1.83)	0.66
ECOG PS≤1	1.13 (0.92 to 1.39)	0.25	0.59 (0.35 to 1.00)	0.05	0.40 (0.22 to 0.74)	**0.003**
Visceral metastases	0.74 (0.62 to 0.89)	**0.002**	2.23 (1.32 to 3.79)	**0.003**	2.53 (1.29 to 4.98)	**0.007**
BMI	1.02 (1.00 to 1.03)	0.05	0.97 (0.93 to 1.01)	0.14	0.91 (0.86 to 0.96)	**0.001**
Albumin	1.20 (1.05 to 1.37)	**0.009**	0.57 (0.43 to 0.75)	**<0.001**	0.49 (0.36 to 0.67)	**<0.001**
Hemoglobin <100 vs ≥100 g/L	0.835 (0.70 to 1.00)	0.05	1.71 (1.11 to 2.65)	**0.02**	1.45 (0.85 to 2.47)	0.18
Creatinine	1.01 (0.92 to 1.11)	0.91	0.83 (0.63 to 1.08)	0.16	0.86 (0.64 to 1.16)	0.32
NLR <5 vs NLR ≥5	1.18 (1.00 to 1.40)	0.06	0.61 (0.40 to 0.94)	**0.03**	0.45 (0.26 to 0.75)	**0.002**
TMB ≥10 mut/Mb vs TMB <10 mut/Mb	3.45 (1.04 to 11.11)	**0.04**	0.42 (0.22 to 0.81)	**0.009**	0.69 (0.03 to 1.43)	0.32
TERT promoter mutation	1.33 (1.08 to 1.65)	**0.01**	0.41 (0.24 to 0.72)	**0.002**	0.53 (0.27 to 1.06)	0.07
MDM2 mutation	1.41 (1.01 to 1.96)	**<0.05**	0.98 (0.46 to 2.08)	0.95	0.72 (0.28 to 1.88)	0.51
CDKN2B mutation	0.94 (0.74 to 1.20)	0.61	1.36 (0.77 to 2.40)	0.29	1.91 (0.98 to 3.73)	0.06

Bold values denote statistical significance at the p < 0.05 level.
BMI, body mass index; NLR, neutrophil to lymphocyte ratio; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TMB, tumor mutation burden; UC, urothelial carcinoma.

Clinical prognostic factors

Favorable baseline performance status (ECOG ≤1) was associated with a longer OS (HR 0.40, 95% CI 0.22 to 0.74, p=0.003) and a trend toward a longer PFS (HR 0.59, 95% CI 0.59 to 1.00, p=0.05) on univariable analysis (table 2). It remained an independent predictor of OS after adjusting for other pretreatment clinical and laboratory variables (HR 0.46, 95% CI 0.23 to 0.90, p=0.03; table 3). On the other hand, the presence of visceral metastases was associated with shorter PFS (HR 2.24, 95% CI 1.32 to 3.79, p=0.003) and OS (HR 2.53, 95% CI 1.29 to 4.98, p=0.007) on univariable analysis, as well as a lower likelihood of response to ICI (OR 0.74, 95% CI 0.62 to 0.89, p=0.002; table 2). The presence of visceral metastases was not associated with ORR or OS on multivariable analysis, although we did observe a trend toward shorter PFS in this patient population (HR 1.97, 95% CI 0.99 to 3.92, p=0.06; table 3). Pretreatment albumin level, NLR <5 and the presence of pure UC histology in a biopsy sample were associated with favorable outcomes on univariable analysis only. No significant associations with treatment outcomes were seen with location of primary tumor or with receiving ICI in the front-line versus postplatinum setting.

Genomic prognostic factors

Genomic profiling results were available for 78 patients (table 1). Patients with available genomic profiling were more likely to have an ECOG performance status score ≤1 relative to patients without genomic profiling; other baseline characteristics were balanced between the two groups. Most assays were performed through FoundationOne or the CLIA-certified institutional UCSF500 platform (n=44 and n=30, respectively). The most commonly altered genes were the *TERT* promoter (61.0%), *TP53* (51.9%), *RB1* (31.2%), *CKDN2A* (28.6%), *CDKN2B* (27.3%), *ARID1A* (23.4%), *ERBB2* (18.2%), *KDM6A* (19.5%), *PIK3CA* (16,9%), *FGFR3* and *MLL2* (13.0% each). Mutations in the *TERT* promoter and *MDM2* genes were associated with clinical outcomes on univariable analysis: specifically, the presence of a *TERT* promoter mutation was associated with increased response rate (OR 1.33, p=0.010) and longer PFS (HR 0.41, p=0.002), while the presence of an *MDM2* mutation was associated with lower response rate (OR 1.41, p=0.045). There was a non-statistically significant trend towards shorter OS in patients with *CDKN2B* mutations (table 2). Other genomic alterations were not associated with clinical outcomes. On multivariable analysis

Table 3 Multivariable analysis of overall survival with prespecified clinical variables and clinical and genomic characteristics with significant findings on univariable analysis (p<0.1)

	Multivariable analysis (clinical and genomic data; n=78)		Multivariable analysis (entire cohort, clinical data only; n=119)	
Characteristics	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Histology (pure UC vs mixed or pure variant histology)	1.09 (0.32 to 3.74)	0.89	0.91 (0.41 to 2.02)	0.81
ECOG PS ≤1	0.38 (0.11 to 1.32)	0.13	0.46 (0.23 to 0.90)	**0.03**
Visceral metastases	2.47 (0.73 to 8.33)	0.14	1.89 (0.75 to 4.79)	0.18
BMI	0.93 (0.84 to 1.02)	0.14	0.98 (0.89 to 1.03)	0.27
Albumin	0.49 (0.18 to 1.32)	0.16	0.55 (0.30 to 1.01)	0.05
Hemoglobin <100 vs ≥100 g/L	0.41 (0.1 to 1.75)	0.23	0.82 (0.31 to 2.14)	0.68
NLR <5 vs ≥5	1.83 (0.50 to 6.74)	0.36	1.05 (0.51 to 2.15)	0.90
TERT promoter mutation	0.30 (0.10 to 0.93)	**0.04**	N/A	N/A
CDKN2B mutation	1.86 (0.55 to 6.26)	0.32	N/A	N/A

Bold values denote statistical significance at the p < 0.05 level.
BMI, body mass index; ECOG, Eastern Cooperative Oncology Group Performance Status; N/A, not available; NLR, neutrophil to lymphocyte ratio; UC, urothelial carcinoma.

performed in patients with available genomic profiling results, the presence of a *TERT* promoter mutation was the only variable predictive of improved PFS (HR 0.38, 95% CI 0.18 to 0.81, p=0.01) and OS (HR 0.32, 95% CI 0.10 to 0.93, p=0.04) (tables 3 and 4). In an exploratory analysis, survival in patients with both favorable genomic (ie, TERT promoter mutation) and clinical (ie, baseline ECOG score ≤1) prognostic factors was compared with that of patients with only one or no favorable prognostic factor (figure 1). Patients with both a *TERT* promoter mutation and baseline ECOG performance status ≤1 (n=31) had a significantly longer median OS compared with the remaining patients (n=29) (21.2 vs 7.5 months, p=0.03).

Tumor mutation burden

TMB assessment was included in some NGS platforms and available in 62 patients. The median TMB among patients with available data was 11 mutations/megabase (mut/Mb; range 1–55). Median PFS was significantly longer in TMB-high (TMB ≥10 mut/Mb, n=38) compared with TMB-low (TMB <10 m/mb, n=24) patients (15.0 vs 3.1 months, p=0.005; figure 2). Median OS was also numerically longer in TMB-high patients (28.2 vs 16.5 months) though this difference was not statistically significant (p=0.20). Compared with TMB-low status, TMB-high status was associated with a higher ORR(OR 3.45, 95% CI 1.04 to 11.11, p=0.043) and longer PFS (HR 0.42, 95% CI 0.22 to 0.81, p=0.009), but not OS (table 1, figure 2). As

Table 4 Multivariable analyses of progression-free survival with prespecified clinical variables and clinical and genomic characteristics with significant findings on univariable analysis (p<0.1)

	Multivariable analysis (clinical and genomic data; n=78)		Multivariable analysis (entire cohort, clinical data only; n=119)	
Characteristics	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Histology (pure UC vs mixed or pure variant histology)	0.92 (0.39 to 2.16)	0.84	1.10 (0.59 to 2.04)	0.77
ECOG PS ≤1	0.83 (0.33 to 2.13)	0.69	0.68 (0.38 to 1.20)	0.19
Visceral metastases	1.87 (0.79 to 4.43)	0.16	1.97 (0.99 to 3.92)	0.06
Albumin	0.65 (0.35 to 1.20)	0.17	0.66 (0.42 to 1.05)	0.08
Hemoglobin <100 vs ≥100 g/L	1.01 (0.40 to 2.54)	0.98	1.22 (0.60 to 2.45)	0.58
NLR <5 vs ≥5	1.12 (0.45 to 2.79)	0.81	0.85 (0.47 to 1.52)	0.57
TERT promoter mutation	0.38 (0.18 to 0.81)	**0.01**	N/A	N/A

Bold values denote statistical significance at the p < 0.05 level.
N/A, not available; NLR, neutrophil to lymphocyte ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; UC, urothelial carcinoma.

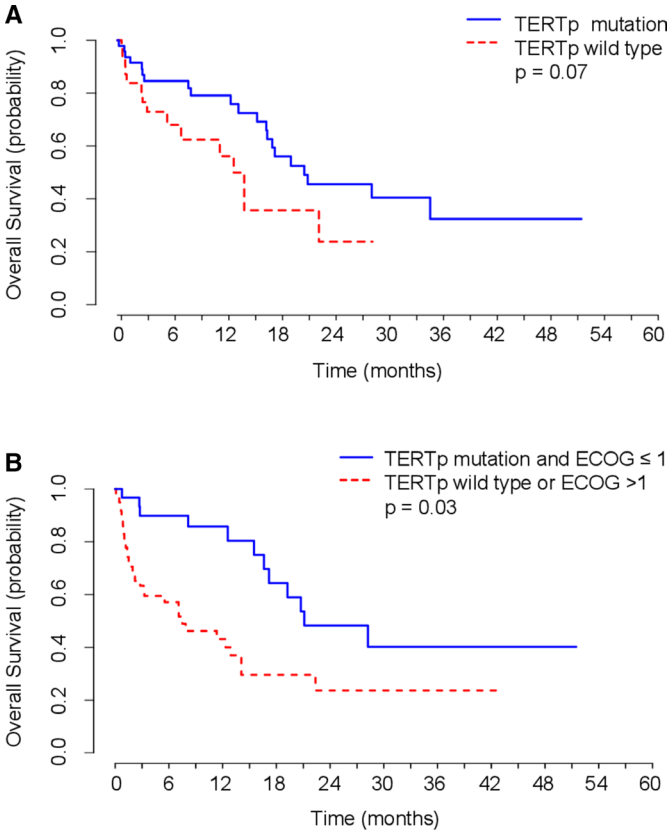

Figure 1 Kaplan-Meier curves of OS (A) in patients with (n=47) vs without (n=31) a *TERT* promoter mutation and (B) in patients with both a *TERT* promoter mutation and favorable pretreatment performance status (ECOG score ≤1; n=31) versus patients with no *TERT* promoter mutation or unfavorable performance status (ECOG score >1; n=29). Log-rank test was used to compare survival between each group. ECOG, eastern cooperative Oncology group; OS, overall survival; TERTp, *TERT* promoter.

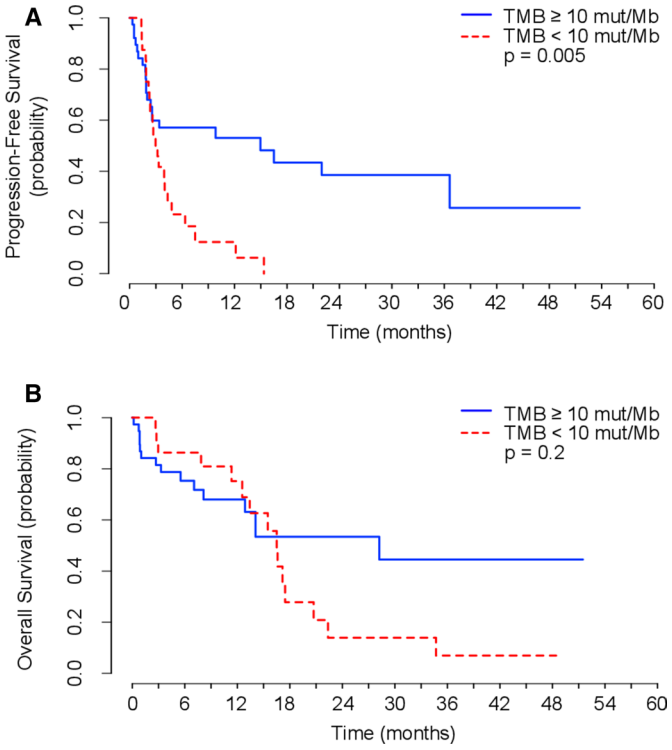

Figure 2 Kaplan-Meier curves of PFS and OS in patients with high (n=38) vs low (n=24) TMB (A, B, respectively) and positive (n=11) vs negative (n=10) PD-L1 expression status (C, D, respectively). Log-rank test was used to compare survival between each group. OS, overall survival; PFS, progression free survival; TMB, tumor mutation burden.

TMB was only available in a subset of patients, it was not included in our multivariable analysis.

We observed a significant interaction between *CKDN2A* and *CDKN2B* alterations and TMB. Specifically, patients with CDKN2A alterations had a significantly lower TMB than patients without (median TMB 7 vs 12 mut/Mb, p=0.04); similarly, patients with *CDKN2B* alterations had a significantly lower TMB compared with wild type counterparts (median TMB 7 vs 12.5 mut/Mb, p=0.02). On the other hand, there was no significant difference in median TMB (11.8 mut/Mb vs 11 mut/Mb) or the incidence of TMB-high status (57.5% vs 66.7%) across patients with or without *TERT* promoter mutations.

PD-L1 expression status

Testing for PD-L1 expression status was performed in 21 patients; PD-L1 was considered positive (Combined Positive Score ≥10) in 11 out of 21 tested cases (52.4%). Compared with PD-L1 negative cases, patients with PD-L1 expression had a significantly longer median PFS (not reached vs 3.36 months, p=0.02) and prolonged median OS (17.5 vs 12.6 months), though the latter did not meet statistical significance (p=0.12; figure 2). Among the subset of patients with positive PD-L1 expression and high TMB (n=7), clinical benefit to ICI treatment was seen for all patients including a CR in three, PR in two, and SD in two patients (ORR 71.4%); median OS was not reached. Given our limited sample size, PD-L1 expression was excluded from our univariable and multivariable analyses, however, 8 of the 11 patients (72.7%) with a positive PD-L1 expression status achieved an objective response to ICI (including 4 CR), while only 2 out of the 10 patients with negative PD-L1 status had PR as best response to ICI, with no observed CRs.

DISCUSSION

In this single-center retrospective cohort of 119 patients with advanced urothelial cancer treated with an ICI, ORR was 29% and median OS was 13.4 months. These results are comparable to the outcomes reported in clinical trials of ICI in aUC published to date.[1–9] With genomic profiling results available in 78 out of 119 patients, this study constitutes one of the larger exploratory analyses of combined genomic, laboratory and clinical characteristics in aUC patients treated with an ICI. The results show that the presence of a *TERT* promoter mutation is associated with improved long-term clinical outcomes, including a significantly longer PFS and OS, even after adjusting for

other baseline characteristics. On the other hand, aside from ECOG performance status, pretreatment clinical and laboratory characteristics were not independently associated with response to ICI or with clinical outcomes. Altogether, these findings suggest that the presence of a *TERT* promoter mutation may represent an important genomic predictive marker of response to ICI treatment in patients with aUC.

TERT promoter mutations increase expression of the *TERT* gene—which encodes the catalytic subunit of the telomerase enzyme.[23] Telomerase activity is suppressed in normal human urothelial cells, and reactivation is thought to constitute an early driver of urothelial carcinogenesis.[24] In line with this, *TERT* promoter mutations are the most common genomic alteration in UC,[25] and were observed in 61% of patients in this cohort. Increased telomerase activity contributes to tumorigenesis by preventing telomere shortening in replicating cancer cells.[24] Telomere length-independent mechanisms of oncogenicity have also been described, including suppression of oncogene-induced and aneuploidy-induced senescence[26] and induction of epithelial-to-mesenchymal transition (EMT).[27] Interestingly, among patients with non-muscle invasive bladder cancer, the presence of a *TERT* promoter mutation was shown to be an independent predictor of nonrecurrence following BCG therapy.[28] However, the prognostic and predictive significance of *TERT* promoter mutations in patients with aUC is not well established. Although an initial study of over 400 bladder cancer samples failed to detect any significant association between *TERT* promoter mutations and clinical outcomes,[29] subsequent analyses have suggested an increased risk of recurrence,[30] distant metastases[31] and decreased survival[32 33] in patients with *TERT* promoter mutations. Importantly, the patients included in these studies were treated with platinum-based chemotherapy regimens. A more recent analysis by Nassar and colleagues evaluated clinical and genomic predictors of response to ICI in a cohort of 62 patients with aUC. However, genomic profiling was performed by exon capture and therefore did not sequence the *TERT* promoter region.[18] Thus, to our knowledge, the impact of *TERT* promoter mutations has not been previously studied in patients with aUC treated with ICIs.[32 33]

In the current analysis, we observed a strong association between *TERT* promoter mutations and superior PFS and OS, even after adjusting for other clinical, laboratory, and genomic variables. Although the prevalence of *TERT* promoter mutations is higher in bladder UC than in upper tract UC, we did not find any significant association between primary tumor location and outcomes in our cohort. Instead, we hypothesize that the presence of a *TERT* promoter mutation may be associated with increased tumor immunogenicity through several potential mechanisms. *TERT* promoter mutations have been shown to promote EMT in various cancer cell lines,[27] which has in turn been associated with increased PD-L1 expression.[34] PD-L1 expression status is associated with increased responses to ICI therapy, but was not assessed in enough patients within this cohort to be included in our multivariable analysis or to assess its relationship with *TERT* promoter mutations. Separately, a study of 398 patients with UC showed that tumors with *TERT* promoter alterations had a significantly higher mutational burden compared with those without *TERT* promoter mutation (median TMB 8 vs 4 mut/Mb; p<0.001), as well as a significantly higher copy number alteration burden.[33] A similar association between *TERT* promoter mutations and higher TMB was recently described in a pan-cancer analysis.[35] The same study also described a longer median OS in a subset of patients with *TERT* promoter mutations treated with anti-CTLA4 agents. Other genomic biomarkers associated with increased TMB (eg, DNA damage repair gene alterations or the presence of an apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) enzyme mutational signature) have been found to predict response to ICI therapy.[18 36] Median TMB in our cohort was numerically slightly higher among patients with a *TERT* promoter mutation compared with wild type patients (11.8 vs 11 m/mb), though this difference was not statistically significant. Interestingly, a recent study of 32 patients with metastatic renal cell carcinoma (RCC) treated with an ICI showed that the presence of a *TERT* promoter mutation was a negative predictor of outcome.[37] In part, these discordant findings underscore cross-cancer differences in mechanisms of antitumor immune response as well as differences in tumor biology. Other biomarkers in aUC, such as PD-L1 expression, have shown similarly conflicting results in predicting response to immunotherapy in RCC[38–40]—while TMB high status in RCC has somewhat surprisingly been associated with inferior outcomes and decreased immune cell infiltration.[41] Ultimately, while our study reports important hypothesis-generating findings, further prospective validation will be needed to confirm the prognostic significance of *TERT* promoter mutation in patients with aUC treated with an ICI and to investigate potential mechanisms that explain this finding.

PD-L1 expression in the tumor and tumor microenvironment has been extensively studied as a biomarker of response and survival in aUC patients receiving ICI. Unfortunately, PD-L1 expression status was not available for most of the patients in our cohort—reflecting standard of care clinical practice prior to mid 2018—and could not be included in our univariable and multivariable analyses. Patients with a PD-L1 CPS ≥10 did in fact have a higher ORR, median PFS and OS compared with patients with a PD-L1 CPS<10. However, ICI treatment still achieved a PR in 2 and SD in 3 out of 10 patients with a CPS <10. Similarly, a higher TMB was associated with improved outcomes in our univariable analysis, yet 6 out of 25 (24%) patients with TMB <10 had an objective response, including 1 CR. Thus, while TMB-high or positive PD-L1 expression status may predict clinical benefit to ICI, there is not currently enough evidence to

justify withholding ICI therapy for aUC patients whose tumors have low TMB or low PD-L1 expression. Interestingly, a recent interim analysis of IMvigor 130 clinical trial suggests that the cooccurrence of high TMB and PD-L1 expression in the tumor may be particularly predictive of a survival benefit with front-line ICI over platinum-based chemotherapy.[19] Consistent with this finding, we observed an ORR of 71.4% (CR 42.9%), among this subset of patients our cohort (n=7), and all of these patients had at least SD as best response to ICI treatment.

Several of the clinical and laboratory markers associated with OS (ECOG performance status, albumin and the presence of visceral metastases) in our univariable analysis have previously been shown to predict survival in patients receiving cytotoxic chemotherapy for aUC.[20 21] Baseline performance status remained predictive of OS on multivariable analysis, and we observed a non-statistical trend toward improved OS in patients with higher albumin. We did not see any independent association between survival and hemoglobin levels or survival and the presence of visceral metastases after adjusting for other clinical variables. While this may in part reflect differences in the underlying mechanism of action of ICI compared with cytotoxic chemotherapy, these results should be interpreted with caution given our smaller sample size. Indeed in another cohort of 62 patients with metastatic UC treated with ICI, the lack of visceral metastases did in fact predict clinical benefit to immunotherapy.[18]

The main limitations of this study are the retrospective nature of our analysis and the relatively small sample size of our cohort—which may have limited the statistical power of our univariable and multivariable analyses. No centralized radiology or pathology review were done as part of this analysis, reflecting the real-world context of this study. We also studied a fairly heterogeneous patient population, including a mix of histological subtypes and patients treated with ICI both in the front-line and treatment-refractory metastatic settings. Tumor mutational profiling was performed on biopsies of primary tumors and distant metastases alike, using several different NGS platforms, yet this experience also reflects real-world clinical practice in most centers. Finally given the lack of a comparison group, it is more challenging to determine whether the variables associated with response and favorable outcome in our cohort were prognostic biomarkers in urothelial cancer patients, or specifically predictive of a clinical benefit to ICI in this patient population. Nonetheless, this study constitutes one of the largest retrospective analyses of combined clinical and genomic factors in aUC patients treated with ICI. Our findings confirm some of the previously reported associations between pre-treatment variables and clinical outcomes, and identify presence of a *TERT* mutation as a novel putative genomic biomarker in this patient population. As for all retrospective analyses, further prospective validation is needed.

CONCLUSION

Consistent with the published literature, this study indicates that only 25%–30% of unselected patients with aUC will respond to checkpoint blockade inhibition in the front-line or platinum-refractory metastatic setting. With the exception of ECOG performance status, established prognostics factors for patients receiving chemotherapy were not associated with OS in this cohort of aUC patients treated with ICIs. On the other hand, the presence of a *TERT* promoter mutation was found to be a novel and independent predictor of improved PFS and OS for aUC patients treated with ICI. The co-occurrence of a *TERT* promoter mutation and favorable pretreatment performance status (ECOG score ≤1) was associated with a particularly good prognosis (median OS of 21.2 months). Whether the presence of a *TERT* promoter mutation is a predictive rather than purely prognostic biomarker in this patient population remains to be determined. Overall, these findings indicate that genomic profiling done through NGS platforms as part of standard clinical practice can provide independent prognostic information among aUC patients treated with ICI and consequently can significantly influence clinical decision making and consideration of patients for clinical trials.

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