Safety, Efficacy, and Biomarker Analysis of Toripalimab in Patients with Previously Treated Advanced Urothelial Carcinoma: Results from a Multicenter Phase II Trial POLARIS-03

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ABSTRACT

Purpose: Immunotherapy offers a second-line option for patients with metastatic urothelial carcinoma (mUC) who failed standard therapy, but the biomarkers for predicting response remain to be explored. This study aims to evaluate the safety, efficacy, and correlative biomarker of toripalimab in patients with previously treated mUC.

Patients and Methods: Patients with mUC received toripalimab 3 mg/kg Q2W. Clinical response was assessed every 8 weeks by an independent review committee per RECIST v1.1. Tumor PD-L1 expression, tumor mutational burden (TMB), and other biomarkers were evaluated.

Results: Among the intention-to-treat population ($n = 151$), 85% of the patients experienced treatment-related adverse event (TRAE) and 20% experienced grade 3 and above TRAE. The objective response rate (ORR) was 26% with a disease control rate (DCR) of 45%. The median duration of response, progression-free survival (PFS), and overall survival (OS) were 19.7 months [95% confidence interval (CI): 13.9–not estimable], 2.3 months (95% CI, 1.8–3.6), and 14.4 months (95% CI, 9.3–23.1), respectively. Both PD-L1$^+$ and TMB-high (10 mutations/Mb as the cutoff) patients had better ORR than PD-L1$^-$ patients (42% vs. 17%, $P = 0.002$) and TMB-low patients (48% vs. 22%, $P = 0.014$), respectively. The TMB-high group also showed better PFS (12.9 vs. 1.8 months, $P < 0.001$) and OS (not reached versus 10.0 months, $P = 0.018$) than the TMB-low group.

Conclusions: Toripalimab has demonstrated encouraging clinical activity in the second-line treatment of mUC with a manageable safety profile. PD-L1 expression and TMB were two independent biomarkers in the study.

Introduction

Patients with metastatic urothelial carcinoma (mUC) have a poor prognosis with a median overall survival (OS) of 15 months and a 5-year survival rate of only around 18% (1). Platinum-based chemotherapy remains the first-line standard of care for mUC. Although about 50% of patients have an initial response to platinum-based chemotherapy (2), the duration of response is typically short-lived with a median progression-free survival (PFS) of 7–8 months (3, 4). Second-line chemotherapy has only limited effect with a response rate of around 10% as a single-agent (5, 6). In recent years, checkpoint inhibitors (ICI) and targeting therapies have changed the standard care for later-line treatment of mUC. Six antibodies against the PD-1 pathway, including pembrolizumab, nivolumab, atezolizumab, durvalumab, avelumab, and tislelizumab, have all received regulatory approval for the second-line treatment of mUC (7–13). The observed objective response rates (ORR) for ICI monotherapy ranged from 15% to 21% in unselected populations and 24% to 28% in the PD-L1$^+$ population (8). In the KEYNOTE-045 phase III trial, a significant OS advantage was observed in the pembrolizumab arm when compared with second-line chemotherapy (7). In contrast, atezolizumab and durvalumab voluntarily withdrew the U.S. indications for prior platinum-treated mUC due to the confirmatory phase III studies had failed to demonstrate survival benefits (14, 15).

In addition to tumor PD-L1 expression, high tumor mutational burden (TMB) has been correlated with better clinical response in patients with various solid tumors including melanoma (16), lung cancer (17), and gastric cancer (18) when receiving immunotherapy, but remained controversial for other indications (19, 20). The utility

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Translational Relevance

Patients with metastatic urothelial carcinoma (mUC) who progress after standard chemotherapy have poor prognosis. Immune-checkpoint inhibitors (ICI) targeting PD-1 or its ligand PD-L1 offer a second-line option. However, the biomarkers for predicting response to ICI therapy remain to be explored. POLARIS-03 is a phase II study in which toripalimab, a humanized IgG4 monoclonal antibody against human PD-1, is used to treat the patients with chemorefractory mUC. This is the largest study that employs whole-exome sequencing and tumor mutational burden (TMB) analysis to evaluate the safety and efficacy of an ICI as a second-line therapy for mUC. It is also the first prospective study for the second-line treatment of urothelial carcinoma with an ICI monotherapy yielding an ORR of more than 40% for PD-L1$^+$ patients. This study demonstrated that the utility of TMB as a biomarker in mUC patients who can predict not only response rate but also survival benefits in response to an ICI therapy.

of TMB to predict better response rate and survival benefit remains to be explored in mUC patients receiving ICI monotherapy.

Additional targeting therapies for the second-line treatment of mUC have been approved by the US FDA in recent years, including erdafitinib for patients with certain *FGFR3* gene mutations or *FGFR2/FGFR3* gene fusions (21) and enfortumab vedotin after platinum and anti–PD-1/PD-L1 therapy (22). In the post-chemotherapy setting, the choice of immunotherapy versus targeting therapy for biomarker-positive mUC patients is yet to be determined.

Toripalimab, a humanized IgG4 monoclonal antibody against human PD-1, received regulatory approval for the second-line treatment of advanced melanoma (23) in December 2018 and for the third-line treatment of recurrent or metastatic nasopharyngeal carcinoma (19) in February 2021 in China. In a phase I study in patients with heavily pretreated metastatic urothelial carcinoma, toripalimab had demonstrated an acceptable safety profile with promising clinical activity (24). Here we report the safety, efficacy, and biomarker analysis of toripalimab in a phase II single-arm study (POLARIS-03) in Chinese patients with previously treated mUC.

Patients and Methods

Patients and study design

This study is a multicenter, single-arm, open-label, phase II clinical trial (NCT03113266) evaluating the safety and clinical activity of toripalimab in patients with locally advanced or metastatic urothelial carcinoma after failure or intolerance to standard chemotherapy. The study protocol and all amendments were approved by the institutional ethics committees of all participating centers. This study was conducted in accordance with the Declaration of Helsinki and the international standards of good clinical practice.

Eligible patients were at least 18 years old with pathologically confirmed locally advanced or metastatic UC who were previously treated with systemic chemotherapy. Patients must have at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 at baseline, with Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate organ and bone marrow function, and willingness to provide consent for biopsy samples. Exclusion criteria included a history of autoimmune diseases or prior anti–PD-1/PD-L1-based immunotherapies.

Treatment and endpoints

Patients received toripalimab 3 mg/kg once every two weeks via intravenous infusion until disease progression, intolerable toxicity, or voluntary withdrawal. Adverse events were monitored continuously and graded according to the NCI Common Terminology Criteria (CTCAE) version 4.0. Radiographic imaging was performed before treatment, then once every 8 weeks in the first year, once every 12 weeks in the second year, and once every 16 weeks in the third year and beyond until disease progression and evaluated by the investigator and an independent review committee (IRC) per RECIST v1.1. Patients who initially developed the progressive disease were allowed to continue therapy if the investigator considered further treatment beneficial to the patients.

The primary endpoints of this study were safety and response rate determined by an IRC per RECIST v1.1. The secondary endpoints included pharmacokinetics and immunogenicity of toripalimab, disease control rate (DCR), duration of response (DOR), PFS, and OS.

PD-L1 expression analysis in tumor biopsies

Archival or fresh tumor biopsy samples were obtained from patients prior to treatment. PD-L1 expression was evaluated by immunohistochemistry (IHC) staining using JS311 antibody with a validated staining assay on the Ventana Benchmark Ultra platform in a central lab (MEDx). JS311 is a monoclonal rabbit anti-human PD-L1 antibody developed for IHC staining (25). A cross-correlation study had been performed among different PD-L1 IHC assays and JS311 showed similar PD-L1 staining patterns and scores as SP263 and 22C3 antibodies in tumor biopsies from various cancer types including UC (Supplementary Fig. S1). PD-L1 positive was defined as the presence of membrane staining of any intensity in ≥ 1% of tumor cells (TC). The cutoff point was selected during method validation prior to the analysis of clinical results. PD-L1 expression on immune cells (IC) was also evaluated. PD-L1 IC$^+$ was defined as immune cell–positive staining ≥ 1%.

TMB analysis

Whole-exome sequencing (WES) was performed with SureSelect Human All Exon V6 kit (Agilent) on tumor biopsies and matched peripheral blood mononuclear cells (PBMC) samples in a central lab (OrigiMed). Genomic alterations including microsatellite stability status, single base substitution (SNV), short and long insertions/deletions (INDEL), copy-number variants, and gene rearrangement and fusions were assessed. The TMB was determined by analyzing somatic mutations including coding base substitution and INDELs per megabase (Mb). A cutoff point of TMB ≥10 mutations/megabase (mut/Mb) was selected for this study based on the recent FDA approval of pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic TMB-high (≥10 mut/Mb) solid tumors that have progressed following prior treatment and have no satisfactory alternative treatment options.

Pharmacokinetics and immunogenicity

Trough PK serum samples were collected every four weeks (every two doses) prior to dosing. An electrochemiluminescence (ECL) method was developed and validated for the detection and quantitation of toripalimab in human serum on an MSD platform as previously described (24). Anti-drug antibodies (ADA) serum samples were collected before the first dose and every four weeks prior to dosing. An ECL method was also developed and validated for the detection of ADA in human serum on an MSD platform. The PK and ADA assays were performed in a central lab (United-Power Pharma).

Statistical analysis

At a one-sided significance level of 0.025, a total of 150 patients could provide 91% power to demonstrate the efficacy of toripalimab at a targeted ORR of 20% versus 10% for the alternative second-line therapy using the Clopper–Pearson method. A sample size of 150 patients was planned for this study.

The safety analysis included all patients who received at least 1 dose of the study drug ($n = 151$). ORR and its 95% exact confidence interval (CI) were determined by the Clopper and Pearson methodology. Fisher exact test was used to compute two-tailed P values from contingency tables. PFS and OS were plotted using the Kaplan–Meier method, with median and corresponding two-sided 95% CI. Statistical analyses were performed with either SAS version 9.4 or the GraphPad Prism software.

Results

Patient population

Between June 2017 and September 2019, 151 patients were enrolled from 15 participating centers (CONSORT diagram; Supplementary Fig. S2). Baseline demographic and clinical characteristics are summarized in **Table 1**. One hundred and thirty-two (87%) patients had visceral metastasis at enrollment, including 50% with pulmonary metastasis, 29% with bone metastasis, and 15% with hepatic metastasis. The primary tumor sites included 47% upper urinary tract and 52% lower urinary tract. Forty-eight (32%) patients had positive PD-L1 expression on tumor biopsies. All patients had received prior systemic chemotherapy, among which 143 (95%) received platinum-based therapy and 8 (5%) received non-platinum chemotherapy. Among platinum-treated patients, 136 patients experienced disease progression on or after platinum treatment, whereas 7 were intolerant to platinum.

Table 1. Summary of baseline demographic and clinical characteristics.

Characteristics	Value	*N* (%)
Age	N	151
	Mean	60.9
	Min, max	28.0, 82.0
Gender	Male	101 (67)
	Female	50 (33)
ECOG	0	66 (44)
	1	85 (56)
TNM stage	IIIb	1 (1)
	IV	150 (99)
Tumor metastasis	Lymph node only	19 (13)
	Visceral	132 (87)
Primary tumor sites[a]	Upper urinary tract	71 (47)
	Lower urinary tract	78 (52)
	Others[b]	2 (1)
Prior lines of treatment[c]	Adjuvant	14 (9)
	1L	105 (70)
	2L+	32 (21)
Prior chemotherapy	Platinum based[d]	143 (95)
	Others	8 (5)
PD-L1 status[e]	Positive	48 (32)
	Negative	96 (64)
	Unknown	7 (5)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; TNM, tumor–node–metastasis staging system.

[a]Upper urinary tract includes renal pelvis and ureter; lower urinary tract includes bladder and urethral canal.

[b]"Other" category included one patient with unknown primary site and one patient with primary sites in both bladder and ureter.

[c]Adjuvant setting included 14 patients who experienced progressive disease within 6 months of the last adjuvant or neoadjuvant chemotherapy. Among 14 patients, 13 received adjuvant chemotherapy and 1 received neoadjuvant chemotherapy. Twelve patients received cisplatin-based chemotherapy, one patient received carboplatin plus nedaplatin, and one patient received nedaplatin.

[d]Among 143 platinum-treated patients, 136 experienced disease progression on or after platinum treatment, whereas 7 patients were intolerant to platinum. Among 143 patients, 112 received cisplatin, 17 received carboplatin and 14 received other platinum therapies.

[e]Positive defined as ≥1% of TCs expressing PD-L1 by JS311 IHC staining.

Treatment-related toxicity

By the cutoff date of September 8, 2020, 12 months after the last enrollment, patients received a median of 8 doses of toripalimab (range, 1–66 doses). The median follow-up was 10.5 months in the intention-to-treat (ITT) population. No new safety signal was identified compared with other ICIs in the same class. One hundred and twenty-eight (85%) patients experienced treatment-related adverse events (TRAE). Common TRAEs (>10%) were listed in **Table 2**. Grade 3 and above TRAEs occurred in 30 (20%) patients, including 27 (18%) patients with grade 3 and 3 (2%) with grade 4 TRAE (Supplementary Table S1). There was no grade 5 TRAE. Permanent discontinuation of toripalimab due to TRAEs occurred in five (3%) patients. Dose delay due to TRAEs occurred in 22 (15%) patients. Two patients developed infusion reactions (one grade 1 and one grade 2), both of which were resolved by symptomatic treatment. Adverse events of special interest included 15 (10%) cases of hypothyroidism, 12 (8%) hyperthyroidism, 4 (3%) abnormal liver function, 2 (1%) interstitial lung disease, 2 (1%) adrenal insufficiency, and 1 (1%) case each of autoimmune hepatitis, hepatic injury, myositis (grade 3), and myocarditis (grade 1).

Antitumor activity

As of September 8, 2020, 81 (54%) patients had died, 13 (9%) remained on treatment, and 57 (37%) discontinued treatment. The median treatment duration was 3.3 months (range, 0.03–30.7 months). Among the ITT population ($n = 151$), 2 CR, 37 PR, and 29 SD were observed for a confirmed ORR of 26% (95% CI, 19–34) and a DCR of 45% (95% CI, 37–53) as assessed by an IRC per RECIST v1.1 (**Table 3** and **Fig. 1**). For patients refractory to prior platinum-based chemotherapy ($n = 136$), the ORR was 27% (95% CI, 20–36). The response rates were similar in patients with primary tumor sites in the upper ($n = 71$) and lower urinary tracts ($n = 78$), 27% versus 24%. The responses were durable as the median DOR was 19.7 months (95% CI, 13.9–NE). The median time to response was 1.8 months (95% CI, 1.7–1.8). For the ITT population, the median PFS and OS were 2.3 months (95% CI, 1.8–3.6) and 14.4 months (95% CI, 9.3–23.1), respectively (**Fig. 2**). Similar response rates were observed as assessed by the investigator per RECIST v1.1 (**Table 3**).

PD-L1 expression in tumor

Tumor biopsy samples were obtained from all 151 patients. PD-L1 IHC staining identified 48 (32%) patients with PD-L1–positive biopsies and 96 (64%) with PD-L1–negative biopsies. Tumor biopsies from seven (5%) patients had either not enough TCs for evaluation or poor tissue quality and were classified as PD-L1 status unknown. Tumor PD-L1^{+} patients, defined by TC-positive staining ≥ 1%, had significantly better ORR and PFS than tumor PD-L1^{-} patients, ORR 42% versus 17%, $P = 0.002$ (**Fig. 3A**); median PFS 3.7 versus 1.8 months, HR = 0.60 (95% CI, 0.41–0.88), $P = 0.001$ (**Fig. 3B**). Furthermore,

Table 2. Common (>10%) TRAEs in the study ($N = 151$).

N (%)	All	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
All TRAEs	128 (85)	31 (21)	67 (44)	27 (18)	3 (2)	0
Blood triglycerides increased	36 (24)	23 (15)	12 (8)	1 (1)	0	0
Proteinuria	29 (19)	9 (6)	20 (13)	0	0	0
Anemia	27 (18)	11 (7)	11 (7)	4 (3)	0	0
Asthenia	26 (17)	21 (14)	4 (3)	1 (1)	0	0
Leukopenia	25 (17)	18 (12)	6 (4)	1 (1)	0	0
Blood cholesterol increased	21 (14)	19 (13)	1 (1)	1 (1)	0	0
Blood creatine phosphokinase increased	21 (14)	12 (8)	7 (5)	1 (1)	1 (1)	0
Blood glucose increased	20 (13)	14 (9)	3 (2)	3 (2)	0	0
Apolipoprotein B increased	17 (11)	17 (11)	0	0	0	0
AST increased	17 (11)	14 (9)	1 (1)	2 (1)	0	0
Rash	17 (11)	11 (7)	5 (3)	1 (1)	0	0
ALT increased	16 (11)	13 (9)	2 (1)	1 (1)	0	0

Note: "Definitely related," "Probably related," and "Possibly related" were classified as "treatment related" AE (TRAE). "Possibly unrelated" and "Definitely unrelated" were classified as "treatment unrelated."
Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase.

tumor PD-L1$^+$ patients had numerically better OS than PD-L1$^-$ patients, median OS 35.6 versus 11.2 months, HR = 0.85 (95% CI, 0.53–1.36). However, the difference was not statistically significant ($P = 0.49$; **Fig. 3C**).

Genomic mutational analysis and TMB

WES was performed on tumor biopsies and paired PBMCs, and the results were available from 135 patients (**Fig. 3D**). The most frequently altered genes identified in this study included *TP53* (58%), *TERT* (51%), *KMT2D* (40%), *CDKN2A* (24%), *CDKN2B* (21%), *KDM2A* (20%), *ERBB2* (17%), *MTAP* (17%), *ARID1A* (15%), *CCND1* (15%), *FGF19* (14%), *PIK3CA* (14%), *FGF4* (13%), *FGF3* (13%), *FGFR3* (13%), *CREBBP* (13%), *E2F3* (12%), *KMT2C* (12%), *NOTCH1* (11%), *ATM1* (10%) and *NECTIN4* (9%; **Fig. 3D**).

Patients with mutations in chromatin remodeler *SMARCA4* ($n = 12$) or tumor suppressor *RB1* ($n = 12$) were associated with significantly better responses to toripalimab than patients with wild-type genes. Patients with either mutation had a higher ORR than patients with wild-type genes, 58% versus 24% (nominal $P = 0.019$, not adjusted for multiple testing). The ORR was 30% (6/20) in patients with *FGFR2/FGFR3* mutations or *FGFR2/FGFR3* gene fusions, and 42% (5/12) in patients with *NECTIN4* genomic alternations (including 11 *NECTIN4* gene amplifications).

TMB was determined by analyzing somatic mutations within the coding region of the human genome. The median TMB value was 4.1 mutations per million base pairs (Mb) in the cohort. Tumor tissues from 27 (20%) patients harbored more than 10 mutations/Mb, including 4 MSI-H patients. Among MSI-H patients, the ORR was 75% (3/4). Using 10 mutations/Mb as the cutoff, patients with TMB-high responded significantly better than patients with TMB low to toripalimab monotherapy, ORR 48% versus 22%, $P = 0.014$ (**Fig. 3A**). Importantly, TMB-high patients also achieved significantly better PFS and OS than TMB-low patients (**Fig. 3E** and **F**): median PFS 12.9 versus 1.8 months, HR = 0.48 (95% CI, 0.31–0.74), $P < 0.001$ and median OS not reached versus 10.0 months, HR = 0.52 (95% CI, 0.31–0.89), $P = 0.018$. Notably, the TMB-high and PD-L1$^+$ were two independent biomarkers, as 20% of total patients, as well as 20% of PD-L1$^+$ patients, were TMB-high (**Fig. 3A**).

Table 3. Clinical efficacy assessed by IRC and investigator in the ITT population per RECIST v1.1.

	IRC ($N = 151$) *n* (%)	Investigator ($N = 151$) *n* (%)
CR	2 (1%)	4 (3%)
PR	37 (25%)	32 (21%)
SD	29 (19%)	33 (22%)
PD	68 (45%)	69 (46%)
NE	15 (10%)	13 (9%)
ORR (%)[a]	39 (26%)	36 (24%)
95% CI	19%, 34%	17%, 31%
DCR (%)[b]	68 (45%)	69 (46%)
95% CI	37%, 53%	38%, 54%

Abbreviations: CI, confidence interval; CR, complete response; DCR, disease control rate; NE, not evaluable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.
[a]ORR = (CR + PR)/total × 100%.
[b]DCR = (CR + PR + SD)/total × 100%.

Pharmacokinetic and immunogenicity

The median trough concentration of toripalimab at steady state was 54.3 μg/mL (range, 14.4–94.4 μg/mL), which was well above the full receptor blocking concentration of 1.5 μg/mL (10 nmol/L; ref. 26). ADA positivity was detected in 10 of 151 (7%) patients after toripalimab treatment. The ORR was 30% in ADA-positive patients, similar to the overall population. There were no significant differences in clinical efficacy and safety between ADA-positive and ADA-negative individuals.

Analysis of additional biomarkers and subgroups

Additional biomarkers or subgroups analyzed for correlation with clinical efficacy included age, gender, baseline ECOG PS score, metastatic status, baseline LDH levels, prior chemotherapy regimens, prior lines of treatments, primary tumor sites, and ADA status (Supplementary Table S2). Among the subgroups, patients with lymph node–only metastasis ($n = 19$) had significantly better ORR than patients with visceral metastasis ($n = 132$), 53% versus 22%, $P = 0.009$.

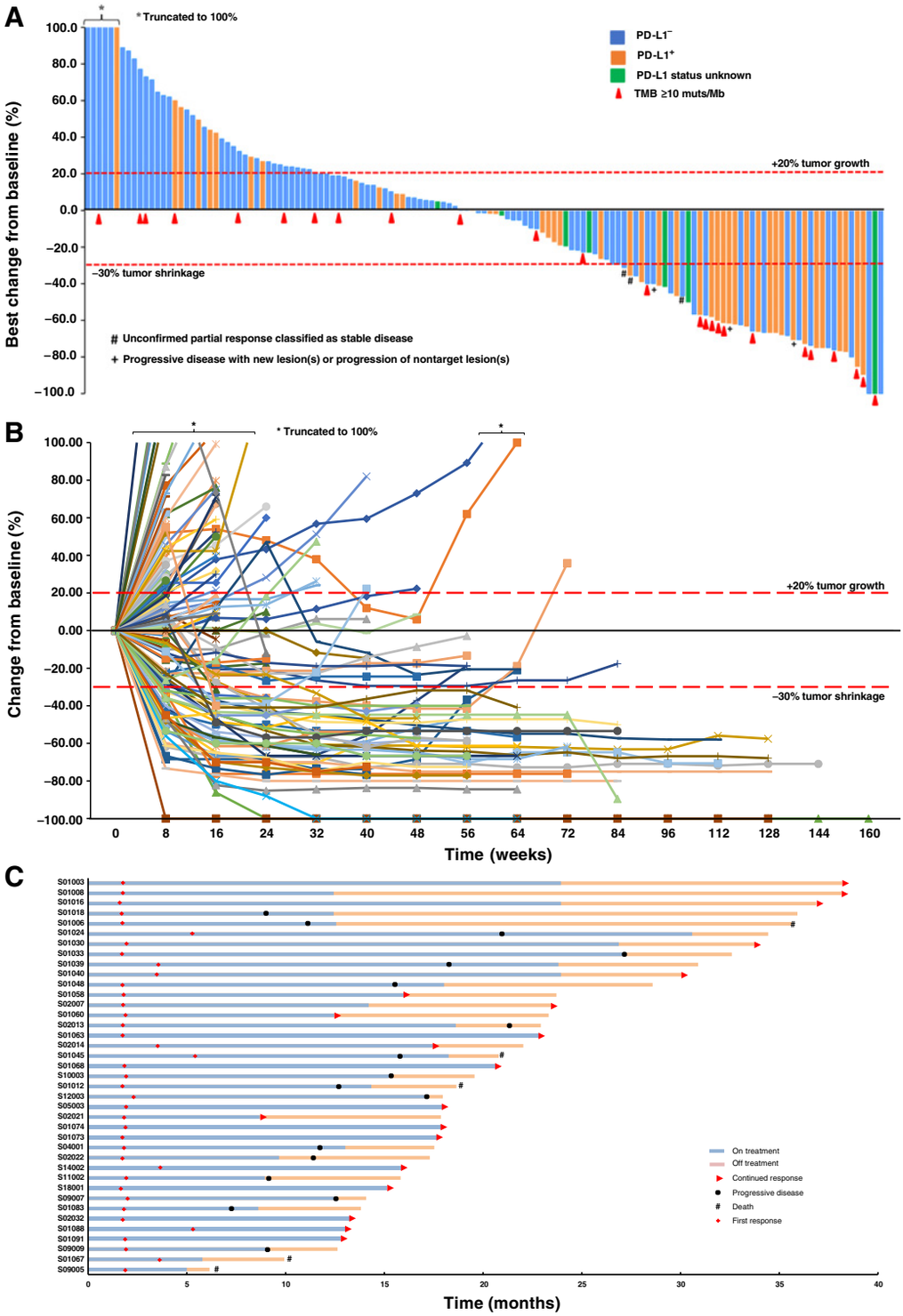

Figure 1.

A, Maximal change in tumor size from baseline assessed by an IRC per RECIST v1.1. The length of the bar represents maximal decrease or minimal increase in target lesion(s). PD-L1–positive status was defined as the presence of membrane staining of any intensity in ≥ 1% of TCs by JS311 IHC staining. TMB was determined by WES. **B,** Change of individual tumor burden over time from baseline assessed by IRC per RECIST v1.1. **C,** Exposure and DOR per RECIST v1.1.

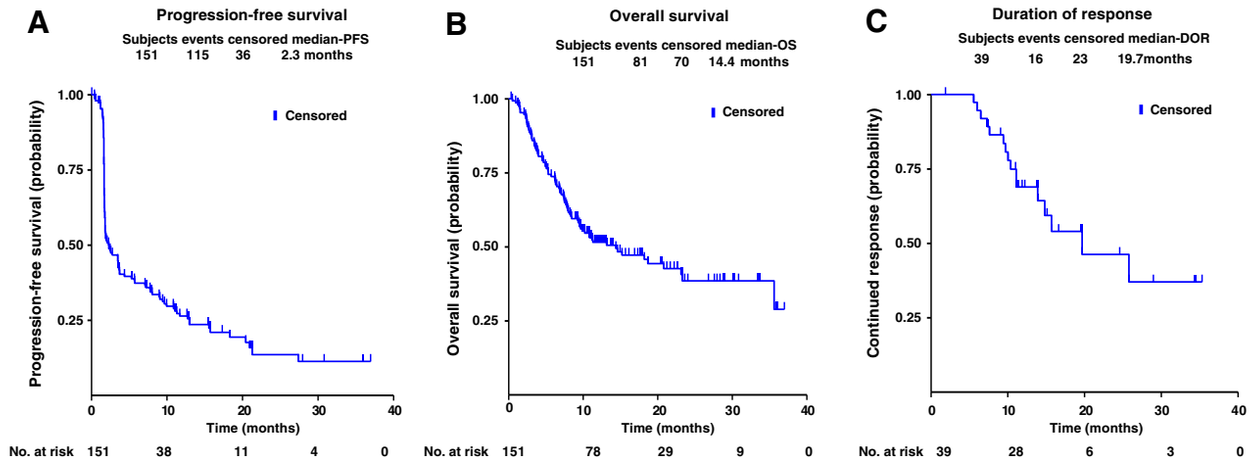

Figure 2.

A, PFS and (**B**) OS of all patients ($n = 151$) in the study. **C,** DOR of responding patients ($n = 39$) in the study. Percentages of survival patients are shown at indicated time points. Censored patients are marked with "|" in the graph. Numbers of patients at risk at indicated time points are shown below the x-axis.

Discussion

After platinum-based first-line treatment, chemotherapy has limited efficacy in patients with mUC. In recent years, ICI and targeting therapies have changed the later-line standard care of mUC. ICI therapies provided ORRs ranged 15%–21% in unselected populations and 24%–28% in the PD-L1$^+$ mUC population. Pembrolizumab, nivolumab, and avelumab were recommended by the NCCN guideline for the second-line treatment of patients with advanced UC. In the KEYNOTE-045 phase III study, pembrolizumab demonstrated a significantly better OS than second-line chemotherapy, 10.3 versus 7.4 months, HR 0.73 (95% CI, 0.59–0.91; $P = 0.002$), with a median PFS of 2.1 months (7). From a single-arm phase II study Checkmate 275 (11), nivolumab achieved a median PFS of 2.0 months and a median OS of 8.7 months. In a phase Ib study (12), avelumab demonstrated a median PFS of 2.7 months and a median OS of 13.7 months. Tislelizumab, another PD-1 inhibitor approved in China, was evaluated in patients with PD-L1–positive urothelial carcinoma who progressed after platinum-containing chemotherapy and induced an ORR of 24%, with a median PFS of 2.1 months and a median OS of 9.8 months (13). In the POLARIS-03 study, toripalimab monotherapy induced a confirmed ORR of 26% in the ITT population and 42% in the PD-L1$^+$ patients. The responses were durable as the median DOR was 19.7 months. In the ITT population, the median PFS and median OS were 2.3 months and 14.4 months, respectively. The response rate and survival results from the POLARIS-03 study were comparable to other anti–PD-1/anti–PD-L1 agents approved for the second-line treatment of mUC.

Using a novel PD-L1 IHC antibody JS311, 48 (32%) patients were identified as TC PD-L1$^+$. TC PD-L1$^+$ patients had significantly better ORR and PFS than TC PD-L1$^-$ patients. We also evaluated IC PD-L1 expression. Tumor IC PD-L1$^+$ patients accounted for 72% (109/151) of patients. Tumor IC PD-L1$^+$ patients also had significantly better ORR than IC PD-L1$^-$ patients, 30% versus 9%. The majority (96%, 46/48) of TC PD-L1$^+$ tumors were also IC PD-L1$^+$. Patients with IC PD-L1$^+$ but TC PD-L1$^-$ tumor ($n = 63$) experienced an ORR of 22%, whereas TC and IC PD-L1 double-negative patients ($n = 33$) had an ORR of only 6% (Supplementary Table S3), indicating TC/IC PD-L1 double-negative UC patients were least likely to respond to toripalimab monotherapy.

In 2020, FDA granted an accelerated approval to pembrolizumab for the treatment of patients with TMB-high (≥10 mut/Mb) advanced solid tumors that have progressed upon prior treatment and with no alternative treatment options. However, a recent study questioned whether TMB status is a reliable universal biomarker for predicting immunotherapy response in all solid tumors. Although TMB-high status is correlated with favorable responses to immunotherapy in selected cancer indications, including melanoma, lung cancer, and urothelial cancer, no correlations were found in other indications, such as breast cancer, prostate cancer, and glioma (20). In this study, we performed WES on available tumor biopsies and evaluated the utility of TMB to predict clinical response. We found a favorable ORR of 48% in patients with TMB over 10 mutations/Mb ($n = 27$). More importantly, the TMB-high group also showed significantly better PFS and OS than the TMB-low group. Notably, among 9 patients with both tumor PD-L1$^+$ and TMB ≥ 10 muts/Mb, the ORR was as high as 78% (7/9). In addition, TMB-high and PD-L1$^+$ were two independent biomarkers in this study. Among patients with TMB-high but PD-L1$^-$ ($n = 18$), the ORR was 33%, indicating the high ORR in the TMB-high population was not driven solely by the PD-L1$^+$ patients.

WES analysis identified divergent mutations from mUC patients in the study. Several recent immunotherapy trials have demonstrated a role for chromatin regulators in response to ICI therapy in selected solid tumors (27, 28). In the POLARIS-03 study, patients with mutations in chromatin remodeler *SMARCA4* ($n = 12$) or *PBRM1* ($n = 10$) had favorable responses to toripalimab (58% ORR and 40% ORR). In addition to PD-L1 and TMB, *SMARCA4/PBRM1* loss-of-function mutations might serve as biomarkers to identify mUC patients for a favorable response to ICI therapy.

In recent years, two targeting therapies have received regulatory approval for mUC patients, including erdafitinib for patients with certain *FGFR3* gene mutations or *FGFR2/FGFR3* gene fusions (21) and enfortumab vedotin as third-line treatment for patients after platinum and anti–PD-1/PD-L1 therapy (22). In addition, HER2-ADC targeting therapy for the second-line treatment of mUC also demonstrated a promising result (29). In the POLARIS-03 study, the ORR was 30%

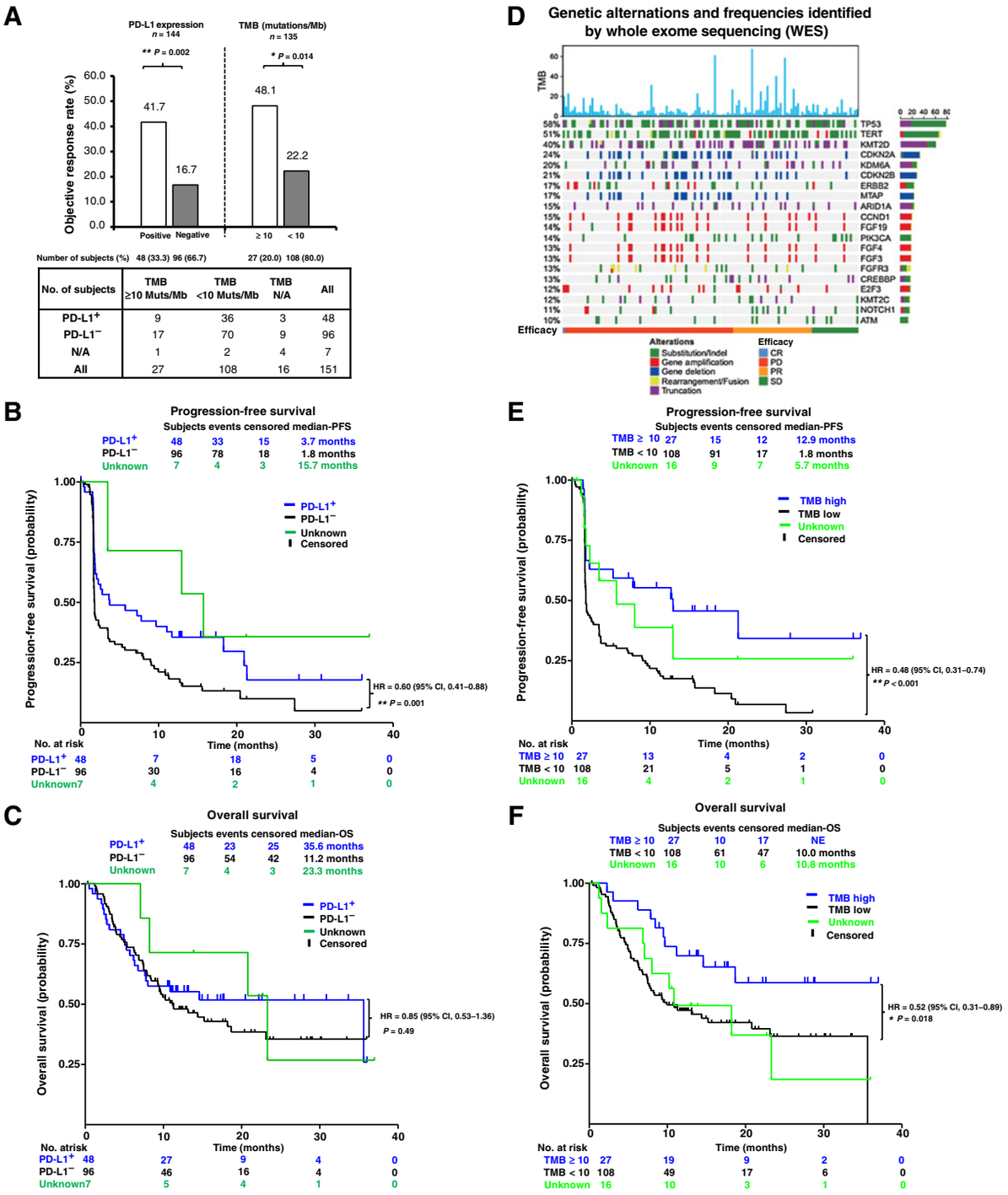

Figure 3.

A, Clinical response in relation to tumor PD-L1 expression or TMB. PD-L1–positive status was defined as the presence of membrane staining of any intensity in ≥ 1% of TCs by JS311 IHC staining. TMB was calculated by total somatic mutations within the coding regions by WES, using 10 mutations per Mb as a cutoff for TMB-high. Number of PD-L1^{+}, PD-L1^{-}, TMB^{high} and TMB^{low} subject are shown in the bottom table. **B,** PFS of PD-L1^{+}, PD-L1^{-} and PD-L1 status unknown patients. **C,** OS of PD-L1^{+}, PD-L1^{-}, and PD-L1 status unknown patients. **D,** Genetic alternations and frequencies identified by WES from 135 available patients. Patients were grouped by clinical responses. **E,** PFS of TMB ≥ 10 Muts/Mb, TMB < 10 Muts/Mb, and TMB unknown patients. **F,** OS of TMB ≥ 10 Muts/Mb, TMB < 10 Muts/Mb, and TMB unknown patients. Percentages of survival patients are shown at indicated time points. Censored patients are marked with "|" in the graph. Numbers of patients at risk at indicated time points are shown below the x-axis. NE, not estimable.

(6/20) in patients with *FGFR3* mutations or *FGFR2/FGFR3* gene fusions, and 42% (5/12) in patients with *NECTIN4* genomic alternations (including 11 *NECTIN4* gene amplifications). Although 23 patients with *ERBB2*/*HER2* genomic alternations had an ORR of 17%, 9 patients with genomic *ERBB2*/*HER2* amplifications had no objective response to toripalimab. In comparison, erdafitinib treatment led to a 40% ORR in *FGFR*-altered UC patients after chemotherapy (21); enfortumab vedotin induced a 44% ORR for mUC in the third-line setting (22), whereas RC48-ADC, a HER2-ADC compound, showed a 51% ORR in $HER2^+$ UC after chemotherapy. Our results indicated that for *FGFR*2/3 biomarker-positive patients, both ICI and targeting therapy should be considered, whereas HER2-ADC might have better response in $HER2^+$ UC patients in the second-line setting. One might speculate the combination of an ICI and a targeting therapy could achieve an even better clinical efficacy in these patient populations.

Based on primary tumor location, urothelial carcinoma is further classified into lower tract urothelial carcinoma (LTUC) originated from the bladder or the urethral canal, and upper tract urothelial carcinoma (UTUC) originated from the renal pelvis or the ureter (30). In the United States, LTUC is the predominant (>90%) subgroup for UC, whereas UTUC accounts for only about 5% of all UC. In contrast, UC in Asia consists of about 50% of LTUC and UTUC each. Given the limited incidence, the response of metastatic UTUC to immunotherapy in the second-line setting was less well characterized than LTUC in previous ICI trials conducted primarily in the Western patient population. Our study found similar response rates in patients with UTUC ($n = 71$) and LTUC ($n = 78$), 27% versus 24%, consistent with a recent report from a real-world study (31). Similar tumor PD-L1–positive rates and percentage of TMB-high (≥ 10 muts/Mb) patients were observed from UTUC and LTUC subgroups in this study, which might explain the similar clinical efficacy in the two subgroups. Toripalimab appeared equally active in UTUC patients with or without *FGFR3* mutations, 27% versus 26%. In contrast, in LTUC patients, toripalimab appeared more active in patients with WT *FGFR3* than patients with *FGFR3* mutations, 29% versus 17%.

As exposure to aristolochic acid is associated with UTUC (32), mutational signature analysis was performed as previously described (33) and detected signatures were compared with COSMIC SBS signature database. The mutational signature of A:T→T:A transversion (A-T Tv) as a result from exposure to aristolochic acid was presented in both UTUC and LTUC with cosine similarity greater than 0.92 (Supplementary Fig. S3A). In addition, we calculated A-T Tv percentage in detected SNVs for individual samples. More UTUC patients were found to carry A-T Tv mutations than LTUC patients (Wilcoxon rank test $P = 0.003$; Supplementary Fig. S3B), which is consistent with previous reports (32, 34). With a threshold of 12% for A-T Tv mutation rate (based on median mutation rate + 2 times median absolute deviation across 135 WES samples), 28% (18/64) patients with UTUC carry high A-T Tv mutation rate comparing to 9% (6/68) among LTUC patients (Fisher exact test, $P = 0.006$). In this study, patients with high A-T Tv mutations had a numerically higher, but not statistically significant ORR than patients with low transversion mutations, 38% versus 25% (nominal $P = 0.21$).

In summary, POLORIS-03 study showed a durable clinical response of toripalimab for previously treated mUC patients with a manageable safety profile. The incidences of grade ≥3 TRAE and discontinuation due to TRAEs were similar to those of pembrolizumab and nivolumab as a second-line treatment for metastatic UC. To the best of our knowledge, this is the first prospective clinical trial for the second-line treatment of UC demonstrating a response rate greater than 40% for $PD\text{-}L1^+$ patients receiving an ICI monotherapy. Here we report, also for the first time, the utility of TMB in patients with mUC to predict not only the ORR and PFS but also OS benefits in response to an ICI therapy. A combination of tumor PD-L1 expression and TMB might serve to best predict clinical response. A confirmatory phase III trial comparing toripalimab versus placebo in combination with chemotherapy as first-line treatment for mUC is ongoing (NCT04568304).

Data sharing statement

The sponsor (Shanghai Junshi Biosciences Co., Ltd.) will consider requests for providing study protocol. Requests for patient-level data from this study can be submitted via e-mail to guoj307@126.com or hyrrenji2@aliyun.com with detailed proposals for approval. A signed data access agreement with the sponsor (Junshi) is required before data sharing.

Data availability statement

The data generated in this study are available within the article and its supplementary data files.

Authors' Disclosures

W. Wang reports personal fees from OrigiMed during the conduct of the study. H. Feng reports personal fees from Topalliance Biosciences Inc during the conduct of the study; personal fees from Topalliance Biosciences Inc outside the submitted work. S. Yao reports grants from Shanghai Junshi Biosciences and personal fees from TopAlliance Biosciences during the conduct of the study; grants from Shanghai Junshi Biosciences and personal fees from TopAlliance Biosciences outside the submitted work. P. Keegan reports personal fees from TopAlliance Biosciences during the conduct of the study; personal fees from Top Alliance Biosciences outside the submitted work; and is an employee of TopAlliance Biosciences, a wholly owned subsidiary of Shanghai Junshi Biosciences. J. Guo reports other support from MSD, Roche, Pfizer, Bayer, Novartis, Simcere Pharmaceutical Group, Shanghai Junshi Biosciences, and Oriengene outside the submitted work. No disclosures were reported by the other authors.

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