Review Article

Comparison of molecular profiles of upper tract urothelial carcinoma *vs.* urinary bladder cancer in the era of targeted therapy: a narrative review

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Background and Objective: Although upper tract urothelial carcinoma (UTUC) shares the histological appearance of urinary bladder cancer (UBC), molecular studies suggest that UTUC and UBC represent two distinct disease entities. However, treatment approaches for UTUC are virtually extrapolated from the evidence on UBC. As targeted drugs—immune-checkpoint inhibitors, fibroblast growth factor receptor inhibitors, and antibody-drug conjugates—target specific molecules, gaining more knowledge about the target-molecular profiles of each drug can help formulate optimal treatment strategies for UTUC.

Methods: This narrative review summarized the subgroup analyses of clinical trials of FDA-approved targeted drugs to explore the differential effects of each targeted drug when administered for UTUC compared to UBC. We focused on the differences in mutation frequency, RNA expression subtype, and therapeutic target protein expressions (specifically PD-L1, Nectin-4, and Trop-2) between UTUC and UBC and discussed their relationship with the efficacy of each targeted drug.

Key Content and Findings: A clinical trial of nivolumab in an adjuvant setting (CheckMate 274) implied that immune-checkpoint inhibitors might be less efficacious in UTUC than in UBC. Genomic and transcriptomic studies suggest that UTUC has a high frequency of *FGFR3* mutations and predominantly shows the luminal papillary subtype, which is immunologically cold with low T-cell infiltration. These findings are consistent with a possible lower response rate to immunotherapy in UTUC than that in UBC. Clinical trials of enfortumab vedotin in a third-line setting (EV201 and EV301) implied that enfortumab vedotin might be less efficacious in UTUC than in UBC. Previous immunohistochemical analyses suggest that UTUC might have a slightly lower rate of Nectin-4 positivity than UBC, indicating that enfortumab vedotin was less efficacious in UTUC than in UBC.

Conclusions: Clinical differences in the effects of targeted drugs for UTUC and UBC may highlight the molecular differences between these diseases. The treatment strategy should be optimized based on further investigation of the molecular characteristics of UTUC.

Keywords: Upper tract urothelial carcinoma (UTUC); targeted therapy; immune-checkpoint inhibitors; next-generation sequencing; immunohistochemistry

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Introduction

Upper tract urothelial carcinoma (UTUC) is a rare malignancy of the renal pelvis or ureter, accounting for approximately 5–10% of urothelial carcinoma (UC), with an estimated annual incidence of 1–2 cases per 100,000 inhabitants (1). While UTUC has a similar histologic appearance to urinary bladder cancer (UBC), it shows distinct characteristics from UBC (2). For instance, UTUC develops in the mesoderm-derived epithelium (3) and is more often invasive than UBC at surgery (1). In addition, UTUC is a Lynch syndrome-associated malignancy and can be induced by aristolochic acid (AA), whereas UBC is rarely associated with Lynch syndrome or AA exposure (4-6). These differences suggest that UTUC and UBC represent two distinct disease entities. Therefore, different clinical management strategies from UBC are required for the treatment of UTUC, but the existing treatment approaches for UTUC are virtually extrapolated from the evidence on UBC; there is a lack of evidence on UTUC, which can be attributed to its low incidence and lower number of cases included in clinical trials compare to UBC.

For decades, systemic treatment for locally advanced or metastatic UC (including UTUC) was limited to platinum-containing chemotherapy (7). However, the treatment landscape has changed significantly with the recent approvals of targeted drugs such as immune-checkpoint inhibitors [programmed cell death 1 (PD-1) or PD-1 ligand 1 (PD-L1) inhibitors (pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab)] (8), fibroblast growth factor receptor (FGFR) inhibitors (erdafitinib), and antibody-drug conjugates (enfortumab vedotin and sacituzumab govitecan). As these drugs target specific molecules, unlike conventional platinum-containing chemotherapy, a thorough understanding of the target-molecule profiles of each drug is essential to developing optimal treatment strategies for UTUC. Knowledge of expression profiles of these therapeutic target molecules is expected to yield precision oncology approaches matched to UTUC.

In the review, we aimed to explore the differential effects of each targeted drug when administered for UTUC *vs.* when administered for UBC. In addition, we explored the current insights on the molecular landscape of UTUC compared to that of UBC to discuss its relationship with the efficacy of each targeted drug. We present the following article in accordance with the Narrative Review reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-457/rc).

Methods

We assessed the main clinical trials of targeted drugs for UC (PD-1/PD-L1 inhibitors: pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab; FGFR inhibitors: erdafitinib; antibody-drug conjugates: enfortumab vedotin and sacituzumab govitecan) on which Food and Drug Administration (FDA)-approved (or withdrawn) indications for each drug are based. In addition, we summarized published subgroup analyses of each trial (including supplementary data) to explore the differential effects of each drug when administered for UTUC *vs.* when administered for UBC.

Next, we reviewed studies which analyzed the mutational landscape of UTUC tissues by next-generation sequencing (NGS) and summarized alteration frequencies of representative genes between UTUC (divided into low-grade and high-grade) and UBC tissues (high-grade) in the studies. In addition, we summarized analyses of RNA expression subtypes of UTUC performed in these studies. Studies without available information on tumor grade or studies focusing on UTUC associated with Lynch syndrome or AA exposure only were excluded from the summary.

Finally, we covered and summarized immunohistochemical studies which analyzed the expressions of therapeutic target proteins (specifically PD-L1, Nectin-4, and Trop-2) between UTUC and UBC. The search strategy is summarized in *Table 1*. The literature search was limited to original English-language studies, and case reports, systematic reviews, editorials, commentaries, and meeting abstracts were excluded.

Comparison of targeted drug efficacy between UBC and UTUC in clinical trials

Although UTUC is a part of UC in cohorts of clinical trials for targeted drugs, no previous trials have specifically

Table 1 The search strategy summary

Items	Specification
Date of search	1st July 2022
Databases and other sources searched	PubMed
Search terms used	"upper tract urothelial carcinoma" AND ("genomic" OR "next-generation sequencing" OR "whole-exome sequencing")
Timeframe	2004–2022
Inclusion and exclusion criteria	Included studies: studies which analyzed the mutational landscape of upper tract urothelial carcinoma tissues by next-generation sequencing with available information on mutational frequency and tumor grade; the literature search was limited to original English-language studies
	Excluded Studies: studies focusing on upper tract urothelial carcinoma associated with Lynch syndrome or aristolochic acid exposure only; case reports, systematic reviews, editorials, commentaries, and meeting abstracts were excluded
Selection process	ET conducted the selection

examined the efficacy of drugs against UTUC. Here, we focused on subgroup analyses of clinical trials of each drug to explore the differential effects of individual drugs administered for UTUC *vs.* those administered for UBC. *Figure 1* summarizes subgroup analyses by primary tumor site in clinical trials on which FDA-approved indications of targeted drugs for UC are based. However, these studies were not designed to compare the treatment efficacy of each drug for UTUC and UBC; UTUC cases are inappropriately fewer than UBC cases, and there is a bias in the backgrounds between the two subgroups. Therefore, the interpretations of subgroup analyses should be considered only as a reference.

Immune-checkpoint inhibitors

Pembrolizumab (anti-PD-1)

Keynote 045 study (second-line setting)

Pembrolizumab was approved by the FDA in May 2017 as a second-line treatment after progression during platinum-based chemotherapy based on the results of the phase 3 Keynote 045 study (9). The Keynote 045 study enrolled 542 cases of advanced UC after progression from platinum-based chemotherapy, of which 76 (14%) cases had UTUC. The subgroup analyses for overall survival (OS) showed that the hazard ratio (HR) was 0.77 [95% confidence interval (CI): 0.60–0.97] for UBC and 0.53 (95% CI: 0.28–1.01) for UTUC (*Figure 1*).

Keynote 052 study (first-line setting)

Pembrolizumab was granted accelerated FDA approval in May 2017 as a first-line treatment for locally advanced or metastatic urothelial carcinoma in patients who are not eligible for any platinum-containing chemotherapy based on results from the phase 2 Keynote 052 study (10,11). The FDA converted this indication to a regular approval in August 2021 based on the results from the phase 3 Keynote 361 study (12).

Keynote 052 study enrolled 369 cases of cisplatin-ineligible patients with advanced UC who had not been previously treated with systemic chemotherapy, of which 69 (19%) cases had UTUC. The subgroup analyses for objective response rate (ORR %) showed that ORR was 28% (95% CI: 23–34%) for UBC and 22% (95% CI: 12–35%) for UTUC (*Figure 1*).

Nivolumab (anti-PD-1)

CheckMate 275 (second-line setting)

Nivolumab received accelerated FDA approval in February 2017 as a second-line treatment after progression on platinum-based chemotherapy based on the results from the phase 2 CheckMate 275 study (13). CheckMate 275 enrolled 270 cases of advanced UC after progression on platinum-based chemotherapy, but no information about the number of UTUC cases was available, and no subgroup analysis was performed.

CheckMate 274 (adjuvant treatment for resected high-risk UC)

Nivolumab was approved by the FDA in August 2021 for adjuvant treatment of patients with resected high-risk UC based on results from the phase 3 CheckMate 274 study (14).

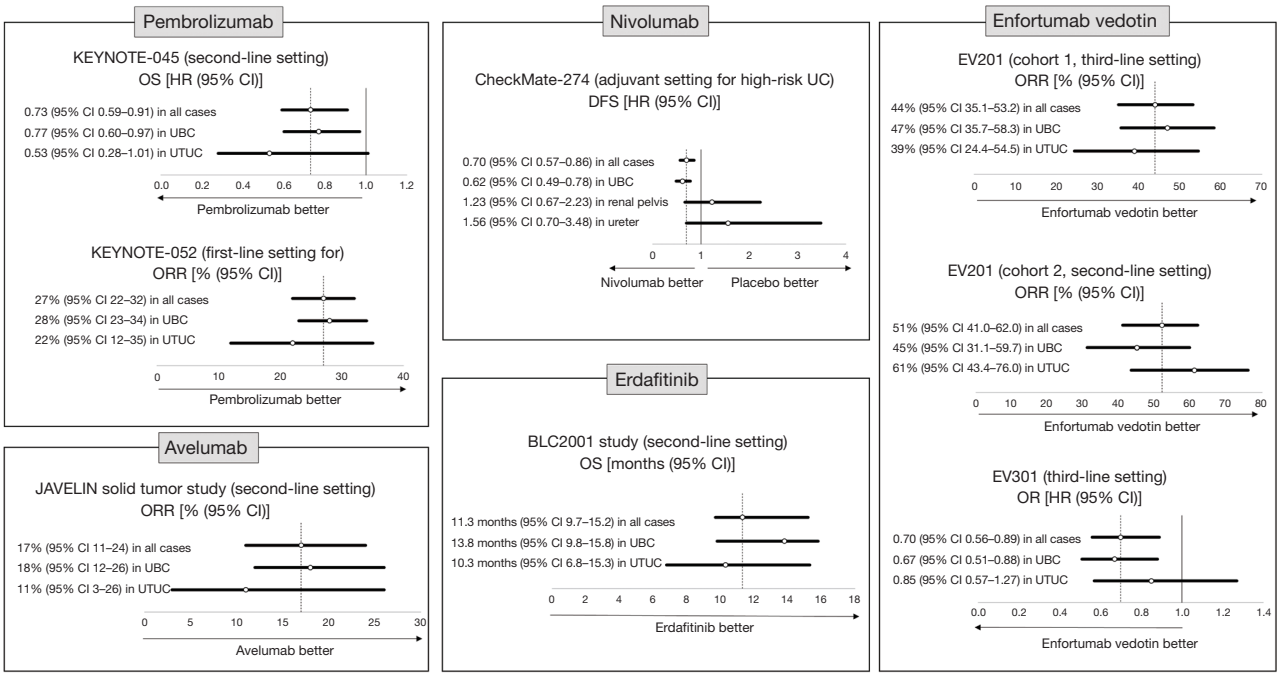

Figure 1 Summary of subgroup analyses stratified by primary tumor site in clinical trials on which FDA-approved indications of targeted drugs for urothelial carcinoma are based. Clinical trials that were not the basis for any FDA-approved indications and clinical trials that did not perform subgroup analyses stratified by primary tumor site were excluded. FDA, Food and Drug Administration; CI, confidence interval; HR, hazard ratio; ORR, overall response rate; OS, overall survival; DFS, disease-free survival; UBC, urinary bladder cancer; UTUC, upper tract urothelial carcinoma.

The CheckMate 274 trial enrolled 709 patients with resected high-risk UC, of which 149 (21%) had UTUC (renal pelvis or ureter). The subgroup analyses for disease-free survival showed that the HR was 0.62 (95% CI: 0.49–0.78) for UBC and 1.23 (95% CI: 0.67–2.23) and 1.56 (95% CI: 0.70–3.48) for renal pelvis carcinoma and ureter carcinoma, respectively (*Figure 1*).

Atezolizumab (anti-PD-L1)

IMvigor210 trial (Cohort 2, second-line setting)

Atezolizumab received accelerated FDA approval in May 2016 as second-line treatment after progression on platinum-based chemotherapy based on the results from the phase 2 IMvigor210 trial (15). However, FDA withdrew the indication as second-line treatment in March 2021, based on the results of the phase 3 IMvigor211 trial (16). The IMvigor210 trial (Cohort2) enrolled 310 cases of advanced UC after progression on platinum-based chemotherapy, of which 65 (21%) had UTUC. ORR was 17% (39/230) for UBC and 8% (5/65) for UTUC.

IMvigor210 trial (Cohort 1, first-line setting)

Atezolizumab received accelerated FDA approval in April 2017 as the first-line treatment for patients with advanced UC who are not eligible for cisplatin-containing chemotherapy (later restricted to patients whose tumors expressed high levels of PD-L1 (17) or who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status, based on the results from the phase 2 IMvigor210 trial (18). The IMvigor210 trial (Cohort1) enrolled 119 cases of advanced UC that were ineligible for cisplatin chemotherapy, of which 33 (28%) had UTUC, but no subgroup analysis was performed in this cohort.

IMvigor211 trial (second-line setting)

The IMvigor211 trial, a phase 3 trial in a second-line setting, did not meet the primary endpoint of improved OS by atezolizumab for patients with high PD-L1 expression l (16).

The IMvigor211 trial enrolled 931 cases of advanced UC after progression on platinum-based chemotherapy, of which 236 (25.3%) patients had UTUC. The subgroup analyses for OS showed that the HR was 0.80 (95% CI:

0.67–0.96) for UBC and 1.12 (95% CI: 0.74–1.70) and 0.86 (95% CI: 0.56–1.32) for renal pelvis carcinoma and ureter carcinoma, respectively. Based on the results of this study, the FDA withdrew the indication as a second-line treatment in March 2021.

Durvalumab (anti-PD-L1)

Study 1108 (second-line setting)

Durvalumab received accelerated FDA approval in May 2017 as a second-line treatment after progression on platinum-based chemotherapy. This approval was based on the updated results from the phase 1 and 2 Study 1108 (19), but the FDA withdrew the indication as second-line treatment in February 2021 based on the results of the DANUBE study (20). Study 1108 enrolled 182 cases of advanced UC after progression on platinum-based chemotherapy, but no information about the number of UTUC cases was available, and no subgroup analysis was performed.

DANUBE study (first-line setting: durvalumab with or without tremelimumab)

The DANUBE study, phase 3 trial, evaluated durvalumab monotherapy compared to chemotherapy as a first-line treatment for advanced UC patients whose tumors express high levels of PD-L1 (20). However, the trial showed that durvalumab monotherapy did not prolong OS compared to chemotherapy in advanced UC with high PD-L1 expression in a first-line setting. The DANUBE study enrolled 690 cases (without tremelimumab), 147 (21.3%) had UTUC, but no subgroup analysis was performed. In addition, the trial also demonstrated that durvalumab plus the CTLA-4 inhibitor tremelimumab did not improve OS compared with chemotherapy in a first-line setting. Based on the results of the DANUBE study, the FDA withdrew the indication as a second-line treatment in February 2021.

Avelumab (anti-PD-L1)

JAVELIN Solid Tumor study (second-line setting)

Avelumab received accelerated FDA approval in May 2017 as second-line treatment after progression on platinum-based chemotherapy based on results from the phase 1b JAVELIN Solid Tumor study (21). The JAVELIN Solid Tumor study enrolled 249 cases of advanced UC after progression on platinum-based chemotherapy, of which 161 cases underwent at least 6 months of follow-up, of which 36 (22%) cases had UTUC. The subgroup analyses for ORR showed that ORR was 18% (95% CI: 12–26%) for UBC and 11% (95% CI: 3–26%) for UTUC (*Figure 1*).

JAVELIN Bladder 100 study (first-line maintenance treatment after chemotherapy)

Avelumab was approved by FDA in June 2020 as a first-line maintenance treatment for patients with locally advanced or metastatic UC that had not progressed with first-line platinum-based chemotherapy based on results from the phase 3 JAVELIN Bladder 100 study (22-24). The JAVELIN Bladder 100 study enrolled 700 cases of advanced UC after progression on platinum-based chemotherapy, of which 187 (26.7%) had UTUC. The subgroup analyses (unpublished data) showed that the HR for OS was 0.62 (95% CI: 0.48–0.80) for UBC and 0.89 (95% CI: 0.58–1.37) for UTUC.

FGFR inhibitors

Erdafitinib

BLC2001 study (second-line setting)

Erdafitinib, a pan-FGFR inhibitor, was approved by the FDA in March 2018 as a second-line treatment for patients with *FGFR3*-altered advanced UC after platinum-based chemotherapy based on the results from the phase 2 BLC2001 study (25,26). The BLC2001 study enrolled 101 cases of previously treated patients who had advanced UC with *FGFR* alterations, of which 25 (25%) had UTUC. The subgroup analyses showed that the median OS was 13.8 months (95% CI: 9.8–15.8) for UBC and 10.3 months (95% CI: 6.8–15.3) for UTUC (*Figure 1*).

Antibody-drug conjugates

Enfortumab vedotin

EV201 (cohort1, third-line setting)

Enfortumab vedotin, a nectin cell adhesion molecule 4 (Nectin-4)-directed antibody and microtubule inhibitor conjugate was granted accelerated FDA approval in December 2019 as a third-line treatment for patients with locally advanced or metastatic UC who previously received platinum-containing chemotherapy and a PD-1/PD-L1 inhibitor based on the results of the phase 2 EV-201 trial (cohort1) (27). The EV-201 trial (cohort1) enrolled 125 patients with advanced UC previously treated with platinum-containing chemotherapy and a PD-1/PD-L1 inhibitor, of which 44 patients (35%) had UTUC. The subgroup analyses showed that ORR was 47% (95% CI: 35.7–58.3%) for UBC and 39% (95% CI: 24.4–54.5%) for

UTUC (*Figure 1*).

EV201 (cohort2, second-line setting)

Enfortumab vedotin was approved by the FDA in July 2021 as a second-line treatment for cisplatin-ineligible patients who had previously received one or more prior lines of therapy based on the results of the phase 2 EV-201 trial (cohort2) (28).

The EV-201 trial (cohort2) enrolled 89 cisplatin-ineligible patients with advanced UC who were previously treated with PD-1 or PD-L1 inhibitors, of which 38 (43%) had UTUC. The subgroup analyses showed that ORR was 45% (95% CI: 31.1–59.7%) for UBC and 61% (95% CI: 43.4–76.0%) for UTUC (*Figure 1*).

EV301 (third-line setting)

Enfortumab vedotin was converted from accelerated FDA approval in 2019 to regular approval in July 2021 as a third-line treatment for patients with locally advanced or metastatic UC who previously received platinum-containing chemotherapy and a PD-1/PD-L1 inhibitor based on results from the phase 3 EV-301 trial (29). The EV-301 trial enrolled 608 patients with advanced UC who received a prior PD-1 or PD-L1 inhibitor and platinum-based chemotherapy, of which 205 patients (33.7%) had UTUC. The subgroup analyses for OS showed that HR was 0.67 (95% CI: 0.51–0.88) for UBC and 0.85 (95% CI: 0.57–1.27) for UTUC (*Figure 1*).

Sacituzumab govitecan

TROPHY-U-01 study (third-line setting)

Sacituzumab govitecan, an anti-Trop-2 monoclonal antibody conjugated to SN-38—an active metabolite of irinotecan—was granted accelerated FDA approval in April 2021 as a third-line treatment for patients with advanced UC who previously received platinum-containing chemotherapy and a PD-1/PD-L1 inhibitor based on results from the phase 2 TROPHY-U-01 trial (30). The TROPHY-U-01 trial enrolled 113 cases of patients with advanced UC who received prior treatment with platinum-containing chemotherapy and either a PD-1 or PD-L1 inhibitor, but no information about the number of UTUC cases was available, and no subgroup analysis was performed.

In summary, several subgroup analyses of clinical trials suggested possible differential effects of targeted agents (especially nivolumab and enfortumab vedotin) between UTUC and UBC. For instance, CheckMate 274 implied that nivolumab might be less efficacious in UTUC than in UBC in an adjuvant setting (14); however, subgroup analysis of other clinical trials, such as the Keynote045 trial, implied that the efficaciousness of pembrolizumab for UTUC appears to be nearly equal or greater than that of UBC (9) (*Figure 1*). In addition, clinical trials of enfortumab vedotin [EV201 and EV301 both in a third-line setting (27,29)] also implied that enfortumab vedotin might be less efficacious in UTUC than in UBC (*Figure 1*).

Comparison between genomic characteristics in UTUC and UBC

The advances in NGS technologies over the past few years have provided a better understanding of the mutational landscape of UTUC.

To date, several studies have analyzed the mutational frequency of UTUC tissues by NGS, and most of them compared mutation frequencies between UTUC and UBC (31-39). It should be noted that mutation frequencies of the UTUC cohort in each study are affected by the proportion of low-grade UTUC cases included because genetic mutation profiles of low-grade and high-grade UC are different. For example, the *FGFR3* mutation frequency has been noted to be higher in low-grade tumors, and the *PT53* mutation frequency is higher in high-grade tumors. Therefore, the gene mutation profile in UTUC needs to be stratified by tumor grade if compared with high-grade UBC.

Sfakianos *et al.* analyzed UTUC tissues (low-grade; n=23, high-grade; n=59) and high-grade UBC tissues (n=102) using the MSK-IMPACT platform and compared mutation frequencies in high-grade UTUC and high-grade UBC (31). They reported that *FGFR3* (36% *vs.* 22%), *HRAS* (14% *vs.* 1%), and *CDKN2B* (15% *vs.* 4%) were more frequently altered in high-grade UTUC, whereas *TP53* (25% *vs.* 58%), *RB1* (0% *vs.* 19%), and *ARID1A* (13.6% *vs.* 27.5%) were more frequently altered in UBC. They also showed that *FGFR3* alterations in high-grade UTUC were mutually exclusive with mutations in *TP53*. Using the same UTUC tissues, Bagrodia *et al.* reported that *TP53/MDM2* alterations were associated with adverse clinicopathological outcomes, whereas *FGFR3* mutations were associated with favorable outcomes (40).

Audenet *et al.* also compared the mutational landscapes of UTUC tissues (low-grade; n=30, high-grade; n=165) to UBC tissues (low-grade; n=26, high-grade; n=428) using the MSK-IMPACT platform and revealed that *FGFR3* (40% *vs.* 26%) and *HRAS* (12% *vs.* 4%) were more significantly altered in UTUC, whereas *TP53* (26% *vs.* 46%), *RB1* (3% *vs.* 20%), and *ERBB2* (8% *vs.* 19%) were more often altered in UBC (32). Furthermore, even after adjusting for the

tumor grade, high-grade UTUC tissues harbored more frequent *FGFR3* alterations than high-grade UBC tissues (31% *vs.* 21%).

Moss *et al.* conducted whole-exome sequencing (WES) in UTUC tissues (low-grade; n=12, high-grade; n=15) (33). They confirmed higher rates of *FGFR3* mutations UTUC (low-grade; 92%, high-grade; 60%) compared to the mutation rate in high-grade UBC in TCGA data (n=404) (13%). They also demonstrated a higher frequency of *TP53* mutations in high-grade UTUC (33%) than in low-grade UTUC (8%).

Lee *et al.* performed targeted sequencing (the Ion Torrent Ampliseq cancer panel v2) to detect frequent somatic mutations and compare the genetic alterations between UTUC (n=31) and UBC (n=61) (34). Although the number of high-grade UTUC and low-grade UTUC in this study is not available, the overall frequency of *FGFR3* mutations in UTUC (13%) was relatively lower than the values reported by Sfakianos *et al.* (54%) (31), Audenet *et al.* (40%) (32), and Moss *et al.* (74%) (33). Lee *et al.* (34) analyzed the Korean cohort, whereas the others analyzed Western patients. This difference in the mutation frequency of *FGFR3* might be attributed to the difference in the race/region profile of the cohorts since *FGFR3* mutation frequencies were reported to be relatively lower in UTUC in Asian patients, particularly Han Chinese patients (3–9%), than in Western patients (36–60%) (41,42). The *TP53* mutation frequency in UTUC (71%) by Lee *et al.* (34) was also markedly higher than in other reports, which might also be due to racial/region differences, including AA exposure. Similarly, Yang *et al.* performed targeted sequencing in UTUC (n=45) and UBC (n=73) tissues from Chinese patients (35). In this study, 21% of patients with UTUC were supposed to harbor AA exposure. Although the number of high-grade UTUC and low-grade UTUC is also not available, the frequency of *FGFR3* mutations in UTUC (20%) was relatively low, as in the Korean cohort by Lee *et al.* (34).

Robinson *et al.* performed WES in high-grade UTUC tissues (n=37) and compared the mutational frequency of UTUC to that of UBC tissues from the TCGA cohort (n=124) (36). They demonstrated that *FGFR3* was more frequently altered in high-grade UTUC than UBC (30% *vs.* 14%).

Nassar *et al.* performed targeted sequencing and compared mutation frequencies in UTUC tissues (n=65, low-grade; n=10, high-grade; n=55) and UBC tissues (n=407, low-grade; n=82, high-grade; n=325) (37). They indicated that *HRAS* mutations were enriched in UTUC (low-grade; 10%, high-grade13%) in comparison with UBC. In addition, *FGFR3* (80% *vs.* 16%) and *KDM6A* alterations (50% *vs.* 20%) were enriched in low-grade UTUC than in high-grade UTUC, whereas the converse applied to *TP53* (0% *vs.* 47%).

Grahn *et al.* performed targeted exome-sequencing in UTUC tissues (low-grade; n=7, high-grade; n=29) (43). They investigated the association between mutations and survival in groups of various grades and stages and found that *HRAS* and *TP53* mutation might be linked to poor prognosis, and *FGFR3* mutations might be linked to a favorable prognosis.

In addition to the mutations mentioned in the above studies, *TERT* promoter mutations are one of the most frequent mutations in both UTUC and UBC (44-46). Fujii *et al.* performed *TERT* promoter sequencing in addition to WES in high-grade UTUC tissues (n=199) (38). They demonstrated that the most frequently affected genes included the *TERT* promoter (49%), *KMT2D* (46%), *CDKN2A* (45%), *FGFR3* (45%), and *TP53* (35%). They also compared the mutational frequency of invasive UTUC (n=81) to that of invasive UBC tissues from the TCGA cohort (n=375). As a result, they reported that *FGFR3*, *CDKN2A*, and *KMT2D* were more preferentially altered in invasive UTUC than in invasive UBC, while *ERBB2* was more frequently mutated in invasive UBC.

Necchi *et al.* analyzed high-grade UTUC tissues (n=479) and high-grade UBC tissues (n=1,984) using the Foundation One platform (39). This is the largest dataset analyzing the mutational landscapes of UTUC *vs.* UBC. They demonstrated that *FGFR3* (26% *vs.* 19%) and *HRAS* (7% *vs.* 3%), and *CDKN2A* (40% *vs.* 35%) was more common in high-grade UTUC than in high-grade UBC, while *TERT* promoter mutations (47% *vs.* 68%) and *TP53* (49% *vs.* 58%) and *RB1* (8% *vs.* 21%) were more common in high-grade UBC.

To summarize these genomic studies in UTUC [except for the reports without tumor-grade information (34,35)], similar mutations were seen in both UTUC and UBC, but the two tumors showed differences in the prevalence of mutations——*FGFR3*, *HRAS*, *CDKN2A*, and *KMT2D* were more frequently altered in high UTUC, whereas *TP53*, *RB1*, *KDM6A*, and *ARID1A* were more frequently altered in high-grade UBC (*Figure 2*). Notably, *FGFR3* mutations are present in a significant proportion of high-grade UTUC tumors as well as low-grade UTUC in comparison with high-grade UBC. Thus, the high frequency of *FGFR3*

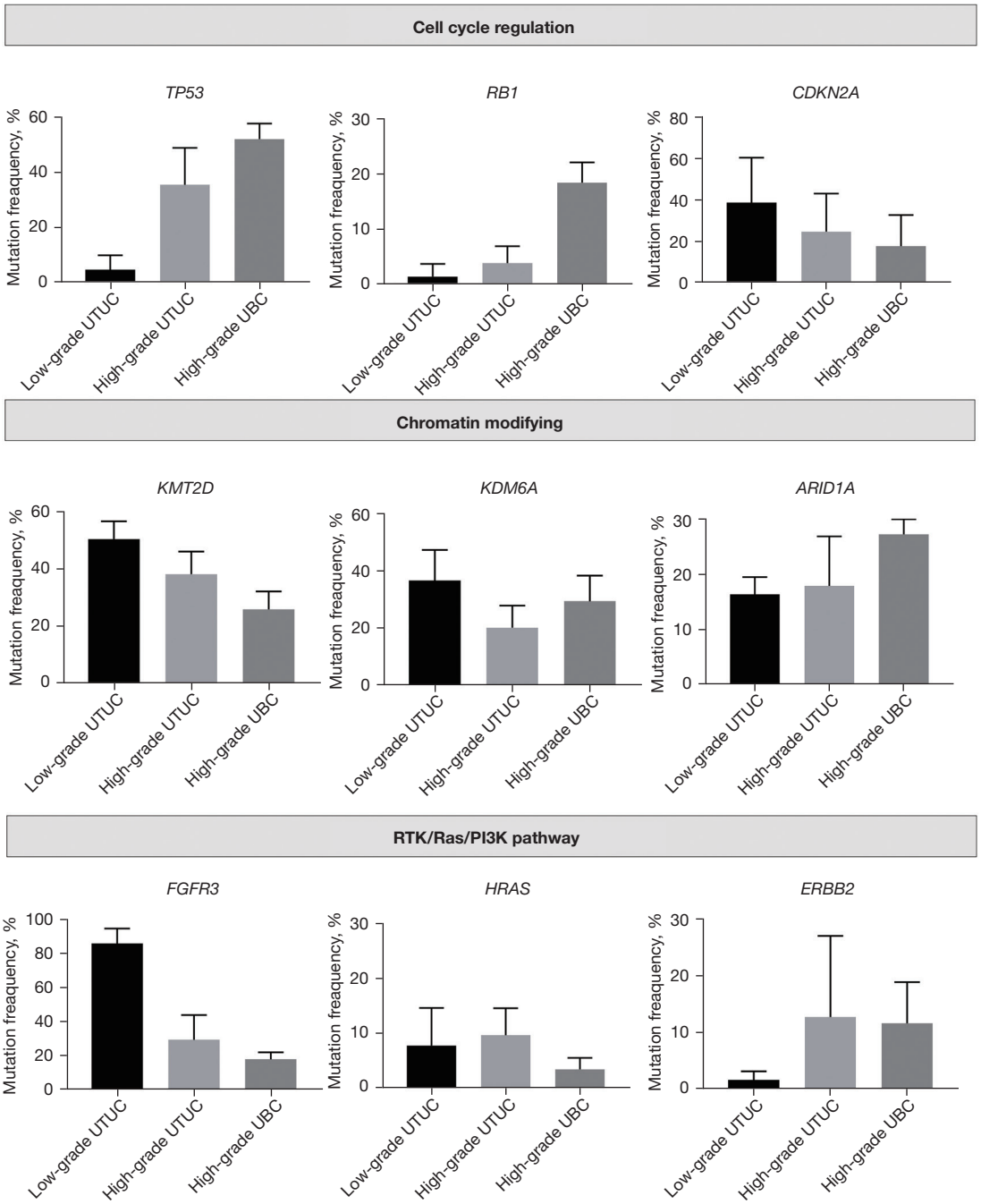

Figure 2 Summarized comparison of alteration frequencies of representative genes in low-grade UTUC, high-grade UTUC, and high-grade UBC tissues analyzed by next-generation sequencing (NGS). Data are expressed as mean and standard deviation (error bar). All studies in which mutation frequencies of UTUC tissues were analyzed by NGS were included. Studies without available information on tumor grade or studies focusing on UTUC associated with Lynch syndrome or AA exposure only were excluded from this figure. AA, aristolochic acid; UBC, urinary bladder cancer; UTUC, upper tract urothelial carcinoma.

mutations is a hallmark of sporadic UTUC. Contrastingly, *TP53* mutations are predominantly found in high-grade UTUC and UBC.

In addition to sporadic mutations, UTUC is also caused by mutations associated with Lynch syndrome and AA exposure (4-6). Patients with Lynch syndrome who have germline mutations in the five MMR genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM* have an increased risk of UTUC than that of UBC. Lynch syndrome-associated UTUC has a higher mutation frequency than in the sporadic UTUC samples but has a similar mutational landscape, and *FGFR3* mutation frequencies are similar to those in sporadic UTUC (4).

AA exposure causes a unique mutational pattern, which differs from that in sporadic UTUC: A>T transversions are significantly enriched at splice sites, with a higher mutational load than in sporadic UTUC (5,6,47). In AA exposure-associated UTUC, *TP53* was frequently mutated (58%), and *TP53* mutation is dominated by A>T transversions. In contrast, *FGFR* mutations were rare (8%) in AA exposure-associated UTUC and did not possess A>T transversions (6).

RNA expression subtypes of UTUC

Several studies performed RNA sequencing in addition to WES of DNA in UTUC and analyzed the RNA expression subtypes of UTUC using unsupervised consensus clustering (33,36,38).

Robinson *et al.* reported that sporadic UTUC shows a lower mutational burden than UBC and predominantly shows the luminal papillary subtype (20/32, 63%), in contrast to the proportion of luminal papillary UBC tumors in the TCGA UBC cohort (35/128; 27%). The luminal papillary subtype is characterized by *FGFR3* gene expression signatures and low immune cell infiltration (36).

Moss *et al.* segregated UTUC samples into four subtypes with unique molecular and clinical features. They demonstrated that clusters characterized by *FGFR3* gene alteration (cluster2 and cluster3, which have 100% *FGFR3* gene alteration) had lower mRNA expression levels of *CD274* (PD-L1) (33).

Fujii *et al.* also conducted gene expression analysis of UTUC through unbiased clustering analysis using RNA sequencing data from 158 UTUC tissues and identified five specific expression subtypes (C1–C5). The C1 subtype showed the highest expression of FGFR3-associated markers and was dominant in the luminal papillary subtype, accounting for most UTUC samples (72%). However, the C3 subtypes, many of which belong to the *TP53/MDM2*-mutated subtype, were classified into the basal-squamous subtype and exhibited high expression of immune-checkpoint molecules, such as PD-L1 (38).

In conclusion, several studies have shown that UTUC predominantly shows the luminal papillary subtype more than UBC (33,36,38). The luminal papillary subtype is characterized by T-cell depletion and may be an immunologically "cold" tumor. Although *FGFR3* mutation itself was reportedly not associated with a response rate to PD-1/PD-L1 inhibitors (but FGFR expression was) (48), the predominance of the luminal papillary subtype in UTUC may afford a possible lower response rate to immunotherapy in sporadic UTUC than that in UBC. On the contrary, UTUC classified into the basal-squamous subtype may express high levels of immune-checkpoint molecules, which might benefit from immunotherapy. However, we previously reported that PD-L1 expression is independent of subtype classification in UTUC based on immunostaining analysis (49), and further studies are required to reveal the PD-L1 expression among molecular subtypes of UTUC.

Comparison of the expression of therapeutic target proteins between UTUC and UBC

As discussed in the section titled "Comparison of targeted drug efficacy between UBC and UTUC in clinical trials", subgroup analyses of clinical trials of targeted therapies have indicated the possibility of differential effects of individual drugs when administered for UTUC and when administered for UBC. These differences may be attributed to the expression status of therapeutic target proteins in UTUC and UBC. Here, we have focused on the differences in the expression status of therapeutic target proteins (specifically PD-L1, Nectin-4, and Trop-2) between UTUC and UBC and discussed the efficaciousness of each targeted drug in UTUC. *Table 2* summarizes the expression status of therapeutic target proteins in UTUC and UBC primary tissues analyzed by immunohistochemical analysis (50-54). We also analyzed the positive rates of each protein in different molecular subtypes of UTUC: luminal (GATA3+/CK5/6–), basal (GATA3–/CK5/6+), and double-negative [considered as p53-like or neuroendocrine-like subtype (GATA3–/CK5/6–)] subtypes, since immunohistochemical staining for GATA3 and cytokeratin 5/6 (CK5/6) is reportedly sufficient to determine molecular subtypes (49,55-57).

Table 2 Expression rates of therapeutic target proteins in UTUC and UBC

Target proteins	UTUC				UBC total	Reference
	Total	Luminal subtype (GATA3+/CK5/6–)	Basal subtype (GATA3-/CK5/6+)	Double-negative subtype (GATA3-/CK5/6–)		
PD-L1	10%	8%	13%	12%	18%	(50,51)
Nectin-4	66%	69%	69%	64%	82%	(50,52)
Trop-2	94%	100%	95%	88%	83%	(53,54)

CK5/6, cytokeratin 5/6; GATA3+, GATA binding protein 3; UBC, urinary bladder cancer; UTUC, upper tract urothelial carcinoma; PD-L1, programmed cell death ligand-1.

PD-L1

Although the value of PD-L1 expression in predicting the effect of PD-1/PD-L1 inhibitors in urothelial carcinoma is still inconclusive, some studies have suggested differences in response according to PD-L1 expression in tumor or immune cells (11,15,23). Therefore, we compared the PD-L1-positive rate (defined as positive when more than 5% of the tumor cell membrane was stained) between UTUC tissues (n=99) (50) and UBC tissues (n=56) (51) with the expectation that it would be a predictive marker of response to PD-1/PD-L1 inhibitors. Notably, the PD-L1-positive rates in the two studies are good comparisons since both UTUC and UBC samples were stained with the same antibody and under the same conditions in the same laboratory, though there is a difference in the race of patients (50,51). Thus, we found that the PD-L1-positive rate was lower in UTUC than that in UBC in immunohistochemical analysis (UTUC, 10%; UBC, 18%) (*Table 2*), but the difference was not statistically significant (P=0.21, Fisher's exact test). If PD-L1 expression on tumor cells is a predictive marker of response to PD-1/PD-L1 inhibitors, the lower PD-L1 positive rate in UTUC than UBC in our analysis is consistent with the results of a subgroup analysis of CheckMate 274 (nivolumab), in which PD-1/PD-L1 inhibitors appear to be less efficacious for UTUC than for UBC. However, this contradicts the subgroup analysis in the Keynote045 trial, in which the efficaciousness of PD-1/PD-L1 inhibitors for UTUC appears to be nearly equal to or greater than that of UBC (9).

In the IMvigor210 trial, higher levels of PD-L1 immunohistochemistry expression on tumor-infiltrating immune cells were associated with a higher response rate to atezolizumab in advanced UC and longer OS, but the PD-L1 expression rate on tumor cells did not show an association with an objective response (15). Thus, the clinical usefulness of PD-L1 as a biomarker in advanced urothelial carcinoma is an area of uncertainty, and it may be difficult to explain the difference in response rates to PD-1/PD-L1 inhibitors between UTUC and UBC solely based on PD-L1 expression on tumor cells. The only FDA-approved indication for PD-L1 expression testing is limited to determining the suitability of atezolizumab as first-line monotherapy in patients with advanced UC who were unfit for cisplatin-containing chemotherapy and have not received prior therapy. In this situation, tumor mutation burden (TMB) is reported to be superior to PD-L1 expression as a biomarker for PD-1/PD-L1 inhibitors (58). In addition to TMB, molecular subtypes were also associated with response to PD-1/PD-L1 inhibitors, suggesting that molecular subtypes differed in their underlying immune biology (15). As mentioned in the previous section about RNA expression subtypes of UTUC, UTUC more predominantly shows the luminal papillary subtype, which is immunologically cold with low T-cell infiltration. This finding may afford a possible lower response rate to immunotherapy in UTUC than that in UBC. Further studies are expected to identify biomarkers for predicting the efficacy of immunotherapy in advanced UC.

Nectin-4

Nectin-4 (encoded by *PVRL4*) is the target protein of enfortumab vedotin, an antibody-drug conjugate for locally advanced or metastatic urothelial carcinoma. Since enfortumab vedotin targets Nectin-4, the expression of Nectin-4 in cancer cells is necessary for its cytotoxic effect, and a correlation between Nectin-4 expression and response to enfortumab vedotin has been demonstrated *in vitro* and *in vivo* (59). Nectin-4 expression was reported in 83% of UBC cases (434/524) (52), while we demonstrated that Nectin-4 expression positivity was found in 68% (67/99)

of UTUC cases in our previous study (50). These findings indicated that UTUC might have a slightly lower rate of Nectin-4 positivity than UBC, which supports the results of a subgroup analysis in the EV-201 (cohort 1) and EV301 trials (27,29) and indicates that enfortumab vedotin was less efficacious in UTUC than in UBC. In contrast, the EV-201 (cohort 2) trial indicated that enfortumab vedotin is more efficacious in UTUC than in UBC (28). Several studies have reported an association between molecular subtype and Nectin4 expression (38,59). Chu *et al.* reported that Nectin-4 expression is significantly enriched in luminal subtypes in muscle-invasive UBC (59). For UTUC, Fujii *et al.* showed that the expression level of *PVRL4*, which encodes Nectin-4, was the lowest in the basal/squamous subtype (38). Tumor mutation burden (TMB) is reported to be superior to PD-L1 expression as a biomarker for PD-1/PD-L1 inhibitors. However, in our analysis, Nectin-4 positivity rates did not differ significantly among the UTUC subtypes (*Table 2*).

Thus, the Nectin4-positive rate in UTUC and the relationship between molecular subtype and Nectin4 expression are topics of debate. The Nectin4-positive rate in UTUC and its association with molecular subtype and actual response rate of EV in clinical practice are expected to be analyzed in future studies.

Trop-2

The trophoblast cell surface antigen 2 (Trop-2, encoded by *TACSTD2*) is the target protein of sacituzumab govitecan, an antibody-drug conjugate for locally advanced or metastatic urothelial carcinoma. A Previous study have indicated that cells overexpressing Trop-2 are highly sensitive to sacituzumab govitecan (60), and a pilot study suggested that high Trop-2 expression in UC was positively correlated with treatment response (53). Therefore, evaluation of the expression of Trop-2 in solid tumors is clinically important to predict the efficacy of sacituzumab govitecan. The Trop-2-positive rate in UTUC was found in 94% (94/99) of UTUC cases in our previous study (54), while it was reported to be 83% in UBC (53). This finding suggests that UTUC also has a high Trop-2-positivity rate and is a treatment option for advanced UTUC and UBC.

Regarding the molecular subtype of UC and Trop-2 expression, Chou *et al.* reported that Trop-2 is highly expressed in most subtypes except in the neuroendocrine subtype (60). Similarly, in our data, Trop-2 positivity rates did not differ significantly among the UTUC subtypes (*Table 2*). In our previous study (54), high Trop-2 expression was associated with a good prognosis in UTUC, and the findings also confirmed that the high *TASCSTD2* expression group still showed a favorable prognosis in gene expression analysis using RNA sequencing data. These findings were inconsistent with those reported for UBC; high Trop-2 expression was associated with increased tumor aggressiveness and poor prognosis. This association between high Trop-2 expression and favorable prognosis in UTUC may be a unique feature of UTUC that differs from UBC; therefore, further studies are expected to improve our knowledge of the differences between UTUC and UBC.

Discussion

In this review, we summarized subgroup analyses of clinical trials of FDA-approved targeted drugs to explore the differential effects of each drug when administered for UTUC *vs.* when administered for UBC. Besides, we summarized the differences in mutation frequency, RNA expression subtype, and therapeutic target protein expressions, specifically PD-L1, Nectin-4, and Trop-2.

Some subgroup analyses of clinical trials suggested possible differential effects of targeted agents; in particular, CheckMate 274 implied that immune-checkpoint inhibitors might be less efficacious in UTUC than in UBC in adjuvant settings (14). Several studies have recently compared mutation frequencies between UTUC and UBC by NGS. Our summary suggested that *FGFR3*, *HRAS*, *CDKN2A*, and *KMT2D* were more frequently altered in high UTUC, whereas *TP53*, *RB1*, *KDM6A*, and *ARID1A* were more frequently altered in high-grade UBC (31-33,36-39). Thus, UTUC has a high frequency of *FGFR3* mutations and is reported to predominantly show the luminal papillary subtype, which is immunologically cold with low T-cell infiltration (36). The feature of UTUC is consistent with a possible lower response rate to immunotherapy in UTUC than that in UBC.

In addition, clinical trials of enfortumab vedotin in a third-line setting (EV201 and EV301) implied that enfortumab vedotin might be less efficacious in UTUC than in UBC (27,29). Previous immunohistochemical analyses suggest that UTUC might have a slightly lower rate of Nectin-4 positivity than UBC (50,52), which supports the results of a subgroup analysis indicating that enfortumab vedotin was less efficacious in UTUC than in UBC.

Thus, clinical differences in the effects of targeted drugs between UTUC and UBC shed light on the potential for

molecular differences between the two diseases, such as molecular subtypes and the status of the target molecule of each drug.

The limitation of this review is the absence of clinical data on the efficacy of individual targeted drugs administered for UTUC compared with those administered for UBC. Needless to say, the results of subgroup analysis should be limited to only a reference because of some factors, such as inappropriately fewer cases of UTUC compared with that of UBC and potential bias in background factors between the two subgroups caused by subgroups not being randomly assigned. Further study is needed to investigate the differences in the efficacy of each drug between UTUC and UBC and its relationship to molecular characteristics in real-world clinical practice.

Summary

This narrative review facilitates a deeper understanding of the clinical differences in the effects of targeted drugs between those administered for UTUC and those for UBC and explores the possibility of existing molecular differences between these two diseases, such as the status of the target molecule of each drug.

Optimizing the treatment strategy based on further investigation of the molecular characteristics of UTUC will gain prominence in the future and can help improve treatment outcomes. Future clinical trials are required to contribute data on the efficacy of targeted drugs for UTUC to drive the treatment decision-making process.

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