Peer Review File

Article Information: https://dx.doi.org/10.21037/tau-22-457

Reviewer A

Comment 1:

- The review includes only a few sources and might not be representative. The way of presentation is not ideal, neither. Some data included is not correct. For example, the frequency of FGFR3 mutation is not so high. I suggest the authors misunderstood some data. I am sorry but this work is limited by a less representative and less convincing results. Especially, the mutation frequencies summarized in Figure-2 might not be true. The FGFR3 mutation rate is not so high in UTUC. And the overall quality of the analysis is poor and includes only a few data

Reply 1:

-Thank you for your meaningful comment.
According to your advice, we have changed the scope to include all studies in which mutation frequencies of UTUC tissues were analyzed by NGS.
We considered that the mutation frequencies of low-grade UTUC and high-grade UTUC should be separated for an accurate comparison with high-grade BCa.
Therefore we summarized mutation frequencies of representative genes between UTUC (low-grade and high-grade) and UBC tissues (high-grade) in these studies in Figure2.
FGFR mutation rate in low-grade UTUC (not high-grade UTUC) is reported to be high; 72% (Fujii et al. 2021), 80% (Nassar et al.2019), 86% (Grahn et al. 2021), 90% (Audenet et al. 2019), 92% (Moss et al. 2017), and 96% (Sfakianos et al.2015).
FGFR mutation rate in high-grade UTUC is not as high as in low-grade UTUC but is reported to be higher than in high-grade UBC; 37% vs. 22% (Sfakianos et al.2015), 60% vs. 13% (Moss et al. 2017), 31% vs. 23% (Audenet et al. 2019), 30% vs.14% (Robinson et al. 2019), and 26% vs. 19% (Necchi et al. 2019).
Thanks to your suggestion, Figure 2. has been revised to provide more accurate information.

Changes in the text:

We have modified our text in the Method section as follows (see Page 7, lines 108 to 11):

Methods

…

Next, we reviewed the previous 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.

We have added new information about mutation frequencies of UTUC tissues analyzed by NGS to the text as follows (see Pages 21 and 22, lines 350 to 382):

Comparison between genomic characteristics in UTUC and UBC

…

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, 45, 46). Fuji 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 = 1984) 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).

Reviewer B

Comment 1:

- I don't fully agree with the premise of UTUC treatment approaches being extrapolated from UBC, especially in regard to systemic treatments. In most RCTs on systemic treatments, urothelial cancer is studied where both UBC and UTUC are included. I would agree that the proportion of UTUC is generally much smaller than UBC (and purposefully so due to caps on UTUC inclusion), so please reconsider this phrasing.

Reply 1:

-Thank you for your meaningful comment.

As you pointed out, most RCTs include both UBC and UTUC. I agree that UTUC treatment approaches are not literally only extrapolated from the evidence on UBC. However, the evidence obtained from these RCTs is almost exclusively on UBC because the proportion of UTUC is generally much smaller than that of UBC.

In this sense, we think that "UTUC treatment approaches are virtually extrapolated from the evidence on UBC.

Changes in the text:

We have modified our text as advised in the ***Introduction*** *(see Page 5, lines 77-82).*

Introduction

…

Molecular studies have recently documented some distinctions between UTUC and UBC, suggesting that UTUC and UBC represent two distinct disease entities (2). Therefore, different clinical management strategies from UBC are required for the treatment of UTUC (3), 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 (1), and lower number of cases included in clinical trials compare to UBC.

Comment 2:

- The methods section would benefit from a more precise description of what the authors did. For instance, you 'summarized subgroup analysis', but please be specific on what was extracted and considered here, and from which sources (these studies tend to have multiple publications sometimes, as well as extensive supplementary files or data presented at conferences). "Trials" is also non-specific. Did you include single arm phase I trials? Or just randomized ones? Please specify the exact inclusion criteria. Since it is a narrative review, I could also understand that you assessed the main phase III study of each drug for each indication, and just used the 'main publication', but then that should also be described.

Reply 2:

-Thank you for your comment.

As you pointed out, we should have described the definition of trial or subgroup analysis we assessed more precisely. We evaluated the main clinical trials of targeted drugs for UC on which each approved (or withdrawn) indication for each drug is based. In addition, we summarized published subgroup analyses of each trial (including supplementary data) in Figure1.

The subgroup analysis of JAVELINbladder100 was not published data (data presented at conferences); therefore, we excluded this from Figure1.

Changes in the text:

We have added a description of the definition of evaluated trial or subgroup analysis to the Methods section (see Page 6-7, lines 101-107).

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 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.

Comment 3:

- Perhaps related to the vague description of how studies were selected, but why is for instance IMvigor-010 not included? This study covers Atezolizumab, and includes UBC and UTUC and reports subgroup HRs for both locations. I know this study was not positive (but so wasn't DANUBE) but that is not mentioned as a criterion (and shouldn't be in my opinion because these still provide relevant information to your research question). What selection is presented in Figure 1? The caption should make clear that this is not all the data that you looked at.

Reply 3:

-Thank you for your comment.

As you mentioned, the IMvigor010 trial evaluated the role of a checkpoint inhibitor for adjuvant treatment of patients with resected high-risk UC. However, the trial did not meet its primary endpoint of improved disease-free survival in the atezolizumab group over observation, and this indication was not approved by FDA. In this section, we assessed the main clinical trials of targeted drugs for UC on which each FDA-approved (or withdrawn) indication for each drug is based. In addition, we summarized published subgroup analyses (including supplementary data) of each trial in Figure1, limited to trials for FDA-approved indications. Therefore, we did not assess IMvigor-010 and did not include its subgroup analysis in Figure1.

Changes in the text:

We add a description of the definition of evaluated trial or subgroup analysis to Methods for better clarity to the readers (see Page 6-7, lines 101-107).

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 FDA-approved (or withdrawn) indications for each drug are based. In addition, we summarized published subgroup analyses (including supplementary data) of each trial (limited to trials for FDA-approved indication) to explore the differential effects of each drug when administered for UTUC vs. when administered for UBC.

In addition, we clarified that Figure1 is limited to subgroup analysis of trials for FDA-approved indication (at the point of July 2022) for each targeted drug (see the caption of Figure 1).

Figure legends

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.

Comment 4:

- Why did you not consider studies that describe NGS data on either UTUC or UBC? Without that, you may be losing important data, and the comparison could be made over study level within the narrative review?

Reply 4:

-Thank you for your comment.

According to your advice, we have changed the scope to include all studies in which mutation frequencies of UTUC tissues were analyzed by NGS.

Most of these studies analyzed mutation frequencies of UBC (high-grade) in addition to UTUC or compared with mutation data from the TCGA database. Therefore, we summarized mutation frequencies of representative genes between UTUC (low-grade and high-grade) and UBC tissues (high-grade) in these studies in Figure2.

However, we excluded two studies without information available on tumor grade from Figure 2 because we considered that the mutation frequencies of low-grade UTUC and high-grade UTUC should be separated for an accurate comparison with high-grade UBC.

Changes in the text:

We have modified the text in the Method section of our manuscript as follows (see Page 7, lines 108 to 114);

Methods

…

Next, we reviewed the previous 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.

We have added new information about mutation frequencies of UTUC tissues analyzed by NGS to the manuscript as follows (see Pages 21 and 22, lines 350 to 382);

Comparison between genomic characteristics in UTUC and UBC

…

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, 45, 46). Fuji 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 = 1984) 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).

Comment 5:

- I think the part where you say the studies where not designed to test subgroup effects, (line 131) with which I totally agree, would benefit from more interpretation guidance for the reader. What can and what cannot be deducted from the subgroup effects? How does the baseline risk within each group influence this? And in your conclusion section, you are somewhat inconsistent with this notion as you do conclude that immunotherapy could be less efficacious in UTUC than UBC (which is not supported by IMvigor-010 by the way).

Reply 5:

-Thank you for pointing this out.

I agree with your comment that it would benefit to describe more interpretation guidance (e.g., specific limitations) for subgroup analyses.

For instance, factors such as" Potential bias in background factors between the two subgroups due to subgroups not being randomly assigned" and "Inappropriately fewer cases of UTUC compared with that of UBC in subgroup analysis" may reduce the reliability of the analysis. Therefore, we added this limitation in the main document.

In addition, we have changed the expression that immunotherapy could be less efficacious in UTUC than UBC to refer only to the result of the subgroup analysis of CheckMate 274.

Changes in the text:

We have modified our text as follows:

(See Page 8, line 127-131)

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

…

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 bias in background factors between the two subgroups. Therefore, the interpretations of subgroup analyses should be considered only as a reference.

(See Page 17, line 287-290)

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

…

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)

Comment 6:

- It is unclear which samples were referred to when comparing PD-L1 expression in UTUC and UBC.

Reply 6:

-Thank you for pointing out the ambiguity in the text.

We compared PD-L1 expression rate of UTUC samples in reference (45) (n = 99, from Japanese patients) and UBC samples in reference (46) (n = 56, from U.S. patients). 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.

Changes in the text:

We have modified our text as follows (see Page 26-27, line 455-462):

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).

Comment 7:

- The authors should refer to the original source for incidence, not the guidelines.

Reply 7:

-Thank you for your comment.

We have changed reference 1 from the guideline to the original source for incidence.

Changes in the text:

We have modified our reference 1 as advised (see the section of ***References****)".*

References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. CA Cancer J Clin 2022;72:7-33.

Comment 8:

- The authors should use the term efficacious instead of effective when referring to effects estimated in RCTs.

Reply 8:

-Thank you for the suggestion. As advised, we have changed the term"effective" in our text to "efficacious" at all instants in the manuscript.

Changes in the text:

We have changed the term"effective" in our text to "efficacious" as advised (see "efficacious" part in the text).

Comment 9:

- The captions of tables/figures could be much more informative. It would help to be able to read them separately from text by giving a quick overview of where the data that is summarized came from.

Reply 9:

-Thank you for your comment.

According to your advice, we added more information to the Figure legends by providing an overview to indicate the source of the summarized data.

Changes in the text:

*We have modified our Figure legends as advised (see the section on **Figure legends**)".*

Figure legends

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.

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.