Peer Review File

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Reviewer A:

Comment 1: An update regarding the available literature has been provided with different recommendations based on contemporary text. However, the reader has been left to decide for himself what one needs to do in a given circumstance when faced with a particular scenario. I recommend based on the available literature, the authors provide their opinion on what is ideal for every histologic variant which may provide an updated and scientific best practice evidence for every variant. A separate table discussing the recommended therapy will need to be included as well.

Reply 1: We have included figure 3 as a decision-making aid while considering treatment options in MIBC with variant histology of urothelial carcinoma with in-text references.

A separate table (table 4) containing recommendations specific to individual variants has been included based on the literature reviewed.

Changes in text: Line 475-478, figure 3 and table 4.

Comment 2: There needs to be a revamp in the section that focuses on immunotherapy. There have been a multitude of papers with respect to immunotherapy which has proved to be a game changer. The authors will need to concentrate on whether these landmark recent studies have taken into account the histologic variants and whether practice can potentially change with their introduction.

Reply 2: The authors have modified the Section on immunotherapy by highlighting the various trials on immunotherapy in variant histology of urothelial carcinoma with references [70][71][72][73]. We discuss the area of unmet medical need and the need for including patients with VH in future clinical trials of neoadjuvant immunotherapy to reflect the real world heterogenous population of MIBC.

Changes in text: Line 425-466. References- 70-73 (line 727-740)

Comment 3: Rather than highlighting every paper in Table 2, I would suggest reduction in the size of the table by grouping together various studies with similar studies for better comprehension.

Reply 3: We have modified table 2 by stratifying the studies based on treatment received and histologic subtype. We believe this will help the reader to grasp the main study findings at a glance. Some of the studies were difficult to classify based on the number of variants they discussed and how heterogenous the study findings were. We have removed any extraneous information not relevant to the research question.

Changes in text: Table 2

Reviewer B:

Comment: This is a commendable effort by authors to look at outcomes of neoadjuvant therapy prior to radical surgery.

The results of their review are presented in the discussion section, but this should be more appropriately placed in a results section.

The discussion section should preferably discuss briefly how to data presented in this systemic review will apply to the clinician on the ground.

Reply: Thank you for commendation and thoughtful comments. This review attempts to address two research questions-

1. What are the pathologic response rates to neoadjuvant treatment prior to RC in variant histology?
2. What are the oncologic outcomes after RC regardless of treatment received?
   We also explore the factors other than variant histology that affect either pathologic response or oncologic outcomes, the so called 'prognostic' factors.

In response to your comment, we have modified the results section to highlight the studies that answer this research question. Some components of the discussion section were also transferred to results section as well. A summary of the findings in the individual studies is presented in table 2. Under table 2, a separate column "Major independent factor(s) other than histologic phenotype affecting response/outcomes analyzed" has been added. Due to the heterogenous nature of these findings, we have adopted a narrative approach at discussing each variant while referencing the results/studies. We have also discussed the nuances of variant histology with regards to molecular subtypes and immunotherapy.

This gives us the bases for including a new figure and table (figure 3 and table 4 respectively) summarizing our findings with recommendations for clinical practice.

Changes in text: Results section- line 138-156, Table 2. clinical practice recommendations- line 475-478, figure 3 and table 4.

Reviewer D

Broad recommendations

Comment 1: While the review presents itself as emphasizing pathologic response rates and oncologic outcomes, much space is given to molecular details of the specific variants. A lot of this detracts from the main point of the review. I recommend limiting discussion of molecular alterations to those specific for a given variant, such as CDH1 mutation in plasmacytoid variant. It otherwise feels arbitrary. For example, it is true the TERT promoter mutation may be useful in diagnosing the microcystic variant, this finding is common all types of bladder cancer, and is also useful in diagnosing the nested variant. This observation also has little to do with the focus of the review.

Reply 1: We appreciate this recommendation, It is true that TERT mutations are found in other variants and in response we have modified the discussion section and figure 2.

Changes in text: Line 290 and figure 2.

Comment 2: The review feels a bit disjointed and many parts are difficult to follow. I recommend revising so each variant is addressed (as you have done), but organize the information as similar as possible in all the sections. Systematic organization would benefit the reader a great deal. I would then add a short summary of findings. You have done something similar on page 10, but new information is raised in this summary. I would treat as a short summary with recommendations, only references information covered in the sections on each variant. I recognize some studies may challenge this approach, such as reference [14] which includes glandular and squamous. Please do your best.

Reply 2: In response to this comment, we have reorganized the studies in table 2 and stratified them according to treatment received and variant subtype. A separate column “Major independent factor(s) other than histologic phenotype affecting response/outcomes analyzed” was

also added to table 2. These are the 'prognostic' factors that were discussed. Some segments of the discussion section were also appropriately transferred to the result section (see line 138-156). Due to the heterogenous nature of the findings, we have adopted a narrative approach in discussing the results to avoid simply rehashing the information in the results rection.

A new segment- clinical practice recommendations with a new figure 3 (summarizing our findings) and table 4 was added to aid in clinical decision making.

Changes in text: Table 2, clinical practice recommendations- Line 475-478, figure 3 and table 4.

Specific recommendations from Reviewer D

Comment 1: The papers uses a slightly inaccurate definition of histologic variant. Many variants are urothelial carcinoma, such as micropapillary. Please change to something like "named histomorphologies distinct from conventional urothelial carcinoma." You could also use "urothelial carcinoma NOS" to reflected the most recent WHO terminology.

Reply 1: The terminology used is well established in pathology practice and literature. In fact, micropapillary, microcystic, plasmacytoid, etc., are described as "variants" in the CAP protocol for bladder cancers (see below). The term variant is also used in the text of the WHO book. Urothelial carcinoma NOS (not otherwise specified) would be more appropriate for urothelial carcinoma that does not exhibit histologic features of any of the variants we have described.

___ Anterior wall
___ Posterior wall
___ Dome
___ Other (specify): _____
___ Cannot be determined: _____

Histologic Type (Note B) (select all that apply)

Urothelial

___ Papillary urothelial carcinoma, noninvasive
___ Papillary urothelial carcinoma, invasive
___ Urothelial carcinoma in situ
___ Urothelial carcinoma, invasive
___ Urothelial carcinoma, nested (including large nested) variant
___ Urothelial carcinoma, microcystic variant
___ Urothelial carcinoma, micropapillary variant
___ Urothelial carcinoma, lymphoepithelioma-like variant
___ Urothelial carcinoma, plasmacytoid / signet ring cell / diffuse
___ Urothelial carcinoma, sarcomatoid variant
___ Urothelial carcinoma, giant cell variant
___ Urothelial carcinoma, poorly differentiated variant
___ Urothelial carcinoma, lipid-rich variant
___ Urothelial carcinoma, clear cell variant

Comment 2: The last sentence of the abstract is too strong. Biomarkers cannot predict response to immunotherapy at this point. Some data suggests predictive value, but nothing is definite.

Reply 2: We recognize the preliminary nature of the findings regarding biomarkers for immunotherapy and have modified the conclusion the abstract accordingly

Changes in text: Abstract- line 51-53, Conclusion- line 494-496.

Comment 3: Line 79 states incidence of variants is 7-81%. This is a bit extreme. I see the reference where you read this, but most careful studies show UC with squamous differentiation is around 20%, and other variants are much less than this. Please modify accordingly.

Reply 3: We have modified this according to the referenced paper.

Changes in text: Line 80-83.

Comment 4: The histomorphologic definitions for subtypes are somewhat inaccurate, or the descriptions are a bit nebulous. For example, micropapillary is described as frequent mitotic figures and nuclear pleomorphism. These findings aren't specific for this variant. Please use the language from the most recent WHO manual to describe the variants.

Reply 4: We have made minor changes to the descriptions used.

Changes in text: Line 171-174

Comment 5: More could be added for outcomes in plasmacytoid UC. For example, a paper by Keck et al. is central to understanding this variant [BMC Cancer. 2013;13;71]. Other features to include in discussion of this variant are its propensity to involve the peritoneum by be greater than stage pT2 at cystectomy.

Reply 5: In response to this, we have included five more studies [38][39][40][41][42] to this review including Keck et al [38] which reported survival outcomes after radical cystectomy and adjuvant chemotherapy in micropapillary and plasmacytoid UC. In the discussion section we have highlighted this propensity for peritoneal metastasis in Line 228,229, 239,240.

Changes in text: Table 2, Keck et al referenced, Line 235,236

Comment 6: Similar to (5), several important references are lacking. A few reviews on this topic could help identify these papers. A challenge in this arena is the variation in naming authors use.

Reply 6: In response to this, we have modified our search strategy and captured an additional five studies that met our inclusion criteria. This being a systematic review we intend to have reproducible search results. The additional studies are referenced- [38][39][40][41][42]

Changes in text: Table 2

Comment 7: The authors focus on two main studies of molecular subtyping of histologic variants. More work has been done in this area, and should be included. The TCGA study is an important one. There are others. These refences should be included in Table 2.

Reply 7: In response to this, we have referenced the contributions of the Cancer Genome Atlas (TCGA) bladder cancer group analysis [66] as it relates to molecular subtypes of the variants. The TCGA study does not report pathologic response rates to neoadjuvant chemotherapy or survival outcomes of interest after RC, so although it has been referenced, it is not included in table 2.

Changes in text: Line 392-394

Comment 8: There are two inaccuracies in conveying results from the study by Li et al. First, the authors use the term “NMI” bladder cancer where “noninvasive papillary urothelial carcinoma” is more accurate. Second, it is suggestion on line 430 that this paper includes data on response to immune checkpoint inhibitors. Treatment with immune checkpoint inhibitors was not reported on for this study. Please clarify this point.

Reply 8: We recognize these inaccuracies and have made corrections accordingly. Li et al reported on “noninvasive papillary urothelial

carcinoma". Our reference to NMIBC is an inference based on the findings on tumor invasive status.

The comment about response to immunotherapy has been modified. Li et al did not report treatment to immune check point inhibitors. The comment about being "immunogenic" is based on the scoring system presented in this study.

Changes in text: line 453-458