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Mutations of METTL3 predict response to neoadjuvant chemotherapy in muscle-invasive bladder cancer

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Alteration of METTL3 Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer
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Dear Dr. Yang,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below. The reviewers have identified several critical issues that need to be addressed properly for the editorial board to alleviate the major revision and reject recommendation by the reviewers. However, we do want to grant

you that opportunity and concurrently stress not to take this task lightly.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jan 12, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: In this study, Yang et al investigated mutations in patients with muscle-invasive bladder cancer that received neoadjuvant chemotherapy. Though neoadjuvant chemotherapy followed by cistectomy is the gold standard of care for MIBC currently, there are few established markers of response for this treatment and investigations into biomarkers are very relevant to advance patient care. The authors gathered two cohorts of patients and performed exome sequencing for a 13 patient discovery cohort from which they selected exclusively occuring mutations in either responders or non-responders to investigate using targeted sequencing in a 20 patient validation cohort. The authors propose four mutations as potentially predictive (CDH9, METTL3 and PTPRH in NAC responders and CCDC141 in non-responders) and focus on METTL3, which is associated with better prognosis in the TCGA cohort.

MAJOR COMMENTS

- Recently, Taber et al (PMID: 32978382) have published a study with extensive molecular profiling, including whole exome, of patients receiving neoadjuvant or first-line chemotherapy. They describe that the SBS5 (ERCC2 related) mutational signature, and particularly BRCA2 mutation were related to response. Their large series presents an opportunity for external validation that would be crucial to lend credibility to the proposed biomarkers in this paper.

- There was no provided table with all identified mutations and I couldn't see any comment on data availability. Will the raw data be deposited in a database and available, at least upon request? Will the processed mutation calling data be provided as a supplement to the paper?

- In Figure 1B, the authors show an overall mutational panel. However, mutations in some of the key genes that have been previously reported as predictive of chemotherapy response in bladder cancer (ERBB2, ERCC2, recently BRCA2) are not shown, though many are mentioned in the text as not significantly different. If they were not encountered, it should be

reported in the text. Additionally, adding clinical information tracks (stage, sex, age) on the patients in this figure can provide a helpful summary in a more integrated way than Table 1.

- Mutational signatures were computed (though I could not find out how they were derived in the methods section), but not really explored. What are the mutation signatures found? Do they relate to response? Do they relate to previously reported mutational signatures, such as the TCGA signatures? As Taber et al found that the ERCC2 mutational signature is predictive of response in their series, this line of investigation is important.

- For the pathway enrichment analysis, it is not clear what gene list was used. Since there were no significant genes differential between responders and non-responders in the discovery cohort, it's unclear what the gene list would be. Mutational signatures can be helpful in this context. It's also highly unusual that DNA repair was not within the results of the enrichment as it is the most well known pathway related to response to chemotherapy not just in bladder cancer.

- The comparison with TCGA mutation frequencies shown in Figure 4 is misleading. The authors conclude that the mutation frequency in responders or non-responders are enriched when comparing to the overall mutation rate in TCGA. However, what they fail to address is that in fact their overall mutation rate (considering 33 as your denominator) is significantly higher than in the TCGA cohort for all of these genes and therefore, the conclusion is misleading. I've included a figure with 10000 bootstraps of 33 samples from the TCGA cohort and the distribution of the mutation frequencies expected when compared to the ones found in this cohort. We see that they are significantly lower in TCGA than in discovery, validation and the pooled ("overall") cohort.

- New biomarker reports should comply with RECIST guidelines. Particularly METTL3 should be assessed in terms of odds ratio using previously established biomarkers and clinical variables.

MINOR COMMENTS

Would it be possible to report variables from the pathological assessment, particularly of the biopsy? Tumor size, grade, infiltration and particularly any variants found could be interesting in this context.

- Increase font size in Figure 2 panels A and C and Supplementary Figure 2 panel B.

- The text could use a revision. The mistakes didn't detract from understanding the message, but there are frequent language inaccuracies in the text.

Reviewer #2: There are no page numbers in the PDF, so I will give page numbers that are displayed in the PDF viewer that I'm using.

Discovery (D) cohort: 7/20 patients excluded due to technical failures in sequencing, so n=13, 5 responders and 8 NR. Validation (V) cohort, Sanger sequencing, 16 path responders, 4 NR. The D and V cohorts are relatively small, and there are large differences between the cohorts

in WES failures, and in the fraction of responders. Together, these factors make comparisons with other cohorts more important. Surprisingly, the comparisons offered are unsatisfying. For example: Page 7, line 1ff: mutated gene frequencies were compared with TCGA 2017, but to my understanding response to treatment was not a parameter in the TCGA work. A second example: page 7 line 43ff: the current cBioPortal lists 12 'Bladder cancer' datasets, but the manuscript does not indicate which of these datasets was compared.

Page 5, line 21: I do not see a method description of how mutational signatures were identified. I do not see that the reported signatures were compared to previous work.

Page 5, line 23: "twenty key mutated genes". Typically 'significantly mutated genes' (SMGs) would be reported. Were the 20 genes SMGs?

Page 14, lines 40ff: the sentence repeats "no high quality variant-supporting reads". This should be corrected.

Page 14, line 48: "The preliminary of somatic indels"? It's unclear what this is trying to say. It should be rewritten.

Page 14, line 49: "After that, germline variants could be effectively removed." Methods state that samples were tumour and matched peripheral blood. Would the authors state more clearly how germline variants were identified.

The samples were presumably frozen (i.e. not FFPE), but I do not see this stated.

Page 14, line 60: "indels represented by only one DNA strand" How were such indels identified, i.e. distinguished from indels present on both strands? Why should these be ignored?

Page 14 line 61: ignore "substitutions located 30 bp around predicted indels". Why should these mutation calls be ignored?

Page 15 lines 1: "false positive associated pseudo gene issues" It's unclear what this is trying to say. Please rewrite this to be clearer.

Page 15 line 39: DAVID, "specifying $p < 0.05$". Was a p value used that was corrected for multiple hypothesis testing?

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Authors' response

Response to Editor's and Reviewers' Comments

Dear Editor-in-Chief Dr. Michal Heger:

Attached please find the revised version of our manuscript entitled "Alteration of *METTL3* Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer" (No. JCTRes-D-20-00137). We appreciated very much for those valuable comments and helpful suggestions from you and the reviewers, which have guided us to significantly improve the quality of our manuscript. We have thoroughly revised the manuscript accordingly, and the changes were highlighted in **blue** in the revised version.

Responses to Editor's comments

Question 1. For your guidance, reviewers' comments are appended below. The reviewers have identified several critical issues that need to be addressed properly for the editorial board to alleviate the major revision and reject recommendation by the reviewers. However, we do want to grant you that opportunity and concurrently stress not to take this task lightly.

Response: Thank you very much for those valuable comments and from you and the reviewers and we have thoroughly revised the manuscript seriously.

Responses to Reviewer #1's comments

Question 1: Recently, Taber et al (PMID: 32978382) have published a study with extensive molecular profiling, including whole exome, of patients receiving neoadjuvant or first-line chemotherapy. They describe that the SBS5 (ERCC2 related) mutational signature, and particularly BRCA2 mutation were related to response. Their large series presents an opportunity for external validation that would be crucial to lend credibility to the proposed biomarkers in this paper.

Response: Thank you very much for this remainder. We check the mutation status of *CDH9*, *METTL3*, *PTPRH* and *CCDC141* identified in the current study with the data from above paper (PMID: 32978382). Unfortunately, we did not found any mutational records of *CDH9*, *METTL3*, *PTPRH* and *CCDC141* in the study of Taber et al..

Similarily, although somatic mutations in DNA damage repair genes (DDR; e.g., *ERCC2*, *ATM*, *RB1*, and *FANCC*) have been correlated with cisplatin-sensitivity in MIBC (PMID: 25096233, 26238431 and 28137924), Taber et al. found no association between DNA damage repair (DDR) gene mutation status and chemotherapy response (Fig. 1f). The differences in races, treatment methods and sample sizes might account for this inconsistency.

Question 2: There was no provided table with all identified mutations and I couldn't see any comment on data availability. Will the raw data be deposited in a database and available, at least upon request?

Response: Sorry for this negligence. All identified mutations in the discovery cohort had been provided as **Supplement Tables 3-6**.

According to the requirements of the ministry's human genetic resources office, we applied to the Ministry of Science and Technology of China for the upload of the raw data. The process is in progress and the raw data could be given upon request in the current stage. (page 16, line 412)

Question 3: Will the processed mutation calling data be provided as a supplement to the paper?

Response: As, suggested, all identified mutations in the discovery cohort had been provided as **Supplement Tables 3, 4, 5, 6, 9, and 10**.

Question 4: In Figure 1B, the authors show an overall mutational panel. However, mutations in some of the key genes that have been previously reported as predictive of chemotherapy response in bladder cancer (ERBB2, ERCC2, recently BRCA2) are not shown, though many are mentioned in the text as not significantly different. If they were not encountered, it should be reported in the text.

-Additionally, adding clinical information tracks (stage, sex, age) on the patients in this figure can provide a helpful summary in a more integrated way than Table 1.

Response: Mutations in some of the key genes that have been previously reported as predictive biomarkers of chemotherapy response in bladder cancer, such as DNA damage repair (DDR) genes (*ERCC2*, *ATM*, *RB1*, and *FANCC*), *FGFR3*, *ERBB2* and *BRCA2*. In this study, *ATM* mutated in 2/21 responders and 0/12 non-responders (Table 1, p = 0.27), *RB1* mutated in 1/5 responders and 2/8 non-responders (Table 1, p = 0.83), and *FANCC* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41). However, the alteration of *BRCA2* was not detected in this study. Furthremore, *FGFR3* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41), *ERBB2* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41). *ERCC2* mutated in 1/5 responders and 1/8 non-responders (Table 1, p = 0.72). The differences in races, treatment methods and sample sizes might account for this inconsistency. (pages 7-8, lines 162-169)

-Detail clinical information has been added as **Supplementary Table 1**.

Question 3: Mutational signatures were computed (though I could not find out how they were derived in the methods section), but not really explored.

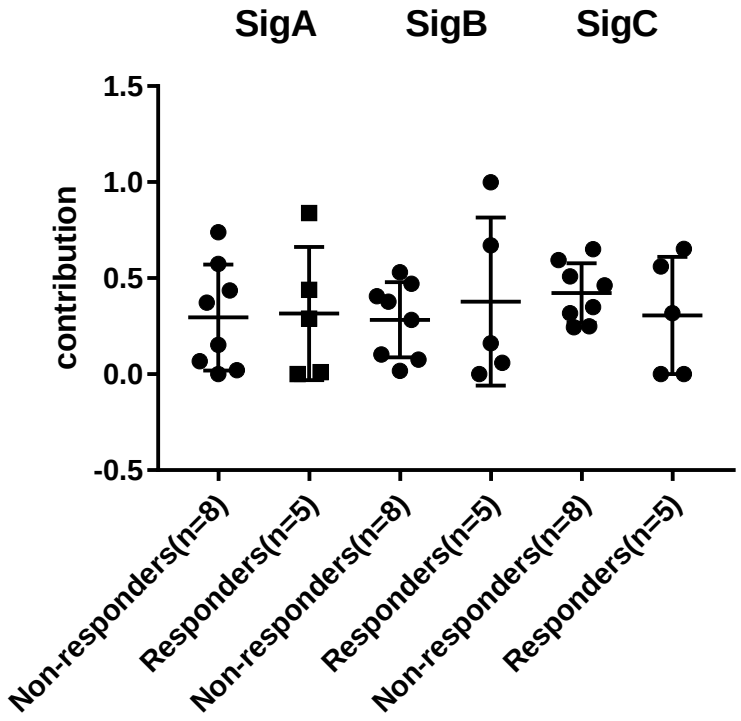
-What are the mutation signatures found?

-Do they relate to response?

-Do they relate to previously reported mutational signatures, such as the TCGA signatures? As Taber et al found that the ERCC2 mutational signature is predictive of response in their series, this line of investigation is important.

Response: In this study, we set out to extract the mutation signature characterizing the mutational processes in the discovery cohort as described before (PMID: 22608084). In briefly, all of the somatic SNV detected in 13 patients were included to calculate the fraction of mutations at each of the 96 mutated trinucleotides. Nonnegative matrix factorization (NMF) was employed to extract biologically meaningful mutational signatures which were displayed by a different profile of the 96 potential trinucleotide mutations. Evaluation of NMF decompositions suggested that three mutational signatures was superior, given to the marginal efficiency of the fourth signature. Also, the relative contributions of the three signatures to each case were estimated. (page 17, lines 452-459)

The C->T/G->A mutation dominated the mutation spectrum in 13 MIBC samples (Supplementary Fig. 2A). And three major mutational signatures (A, B and C) were identified in 13 MIBC samples (Supplementary Fig. 2B and 2C, **Supplementary Table 7**). Refer to Signatures of mutational processes in Human Cancer (https://cancer.sanger.ac.uk/cosmic/signatures). The three signatures A, B and C were similar to Single Base Substitution (SBS) Signature 5, SBS Signature 2 and SBS Signature 6, respectively (Supplementary Table 7). Specifically, the contribution of each signature was calculated for each group, and none signature was significantly enriched in nonresponders or responders (Supplementary Table 8). (pages 6, lines 132-140)



-According to TCGA transcriptional subtypes of BC, all the samples were divided into Luminal subtypes (n = 26) and Basal subtypes (n = 7). The Luminal subtypes or Basal subtypes was not associated with response to NAC (Table 1, p = 0.687). (page 6, lines 116-119)

Question 4: For the pathway enrichment analysis, it is not clear what gene list was used. Since there were no significant genes differential between responders and non-responders in the discovery cohort, it's unclear what the gene list would be. Mutational signatures can be helpful in this context.

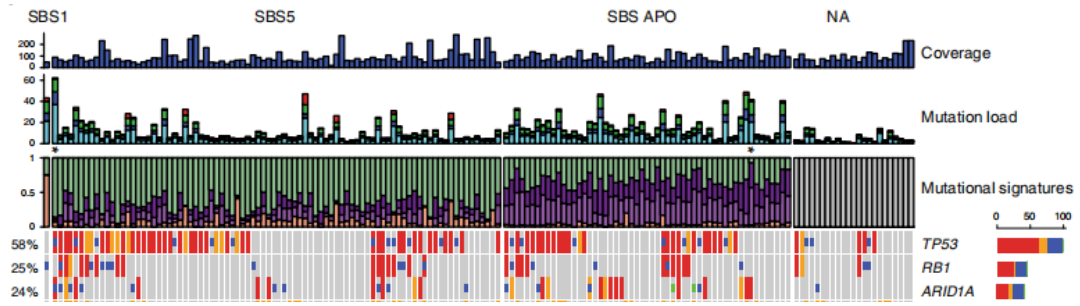
-It's also highly unusual that DNA repair was not within the results of the enrichment as it is the most well known pathway related to response to chemotherapy not just in bladder cancer.

Response: In the pathway enrichment analysis, the exclusively altered genes in the responder group or nonresponder group were chosen. In the revision process, the p value was corrected for multiple hypothesis testing. However, the corrected p values are ≥ 0.05. Thus, the Pathway enrichment section has been deleted from the manuscript. Sorry for this negligence.

Question 5: The comparison with TCGA mutation frequencies shown in Figure 4 is misleading. The authors conclude that the mutation frequency in responders or non-responders are enriched when comparing to the overall mutation rate in TCGA. However, what they fail to address is that in fact their overall mutation rate (considering 33 as your denominator) is significantly higher than in the TCGA cohort for all of these genes and therefore, the conclusion is misleading. I've included a figure with 10000 bootstraps of 33 samples from the TCGA cohort and the distribution of the mutation frequencies expected when compared to the ones found in this cohort. We see that they are significantly lower in TCGA than in discovery, validation and the pooled ("overall") cohort.

Response: In the discovery corhort of this study, *TP53* altered in 7 out of 13 samples (54%), *RB1* altered in 3 out of 13 samples (20%), and *ARID1A* mutated in 2 out of 13 samples (15%). In the study of Taber et al. (PMID: 32978382), *TP53*, *RB1* and *ARID1A* mutated with a frequency of 58%, 25%, and 24%, respectively. In TCGA cohort, *TP53*, *RB1* and *ARID1A* mutated with a frequency of 52%, 21%, and 27%, respectively.

Taber et al



TCGA

Taken together, the mutation frequencies of the genes from this study, the study of Taber et al. (PMID: 32978382) and TCGA are similar. The exclusively altered genes in the response group or non-response group displayed elevated mutation frequencies in the response group or non-response group specifically. Furthermore, the study of Groenendijk FH, et al. (PMID: 25636205) and our previous study (PMID: 29941343) also applied the similar comparison to distinguish the biomarkers predicting the response to NAC in MIBC. Thus, the conclusion of this study is truthful.

Question 6: New biomarker reports should comply with RECIST guidelines. Particularly METTL3 should be assessed in terms of odds ratio using previously established biomarkers and clinical variables.

Response: Thank you for your suggestion. In the study of Plimack et al. (PMID: 26238431), the authors found that genomic alterations in the DNA repair genes *ATM*, *RB1*, and *FANCC* predict response and clinical benefit after cisplatin-based chemotherapy for MIBC. In the current study, the somatic mutation of *METTL3* could be a potential prediction for the pathological response to NAC in MIBC patients. The number of patients in responders and nonresponders acquiring mutated genes were listed below, the odds ratio of discovery and validation cohorts had been compared. However, the odd ratio of discovery and validation

cohorts couldn't be calculated, resulting from that the number of nonresponder patient acquiring altered *METTL3* is zero. In future study, more patients could be recruited to further demonstrated that whether the mutation of *METTL3* serves as an ideal biomarker for the pathological response to NAC in MIBC patients.

Discovery cohort	This study	The study of Plimack et al.
Mutation of genes	*METTL3*	*ATM, RB1, and FANCC*
Responders	2	13
Nonresponders	0	0
Validation cohort		
Responders	6	7
Nonresponders	0	2

$$\text{Odds ratio} = \frac{Responders(this\ study) \Big/ Nonresponders(this\ study)}{Responders(Plimack\ et\ al.\ study) \Big/ Nonresponders(Plimack\ et\ al.\ study)}$$

Question 7: Would it be possible to report variables from the pathological assessment, particularly of the biopsy? Tumor size, grade, infiltration and particularly any variants found could be interesting in this context.

Response: As suggested, detail clinical information has been added as **Supplementary Table 1**. Tumor size information is not available for this cohort.

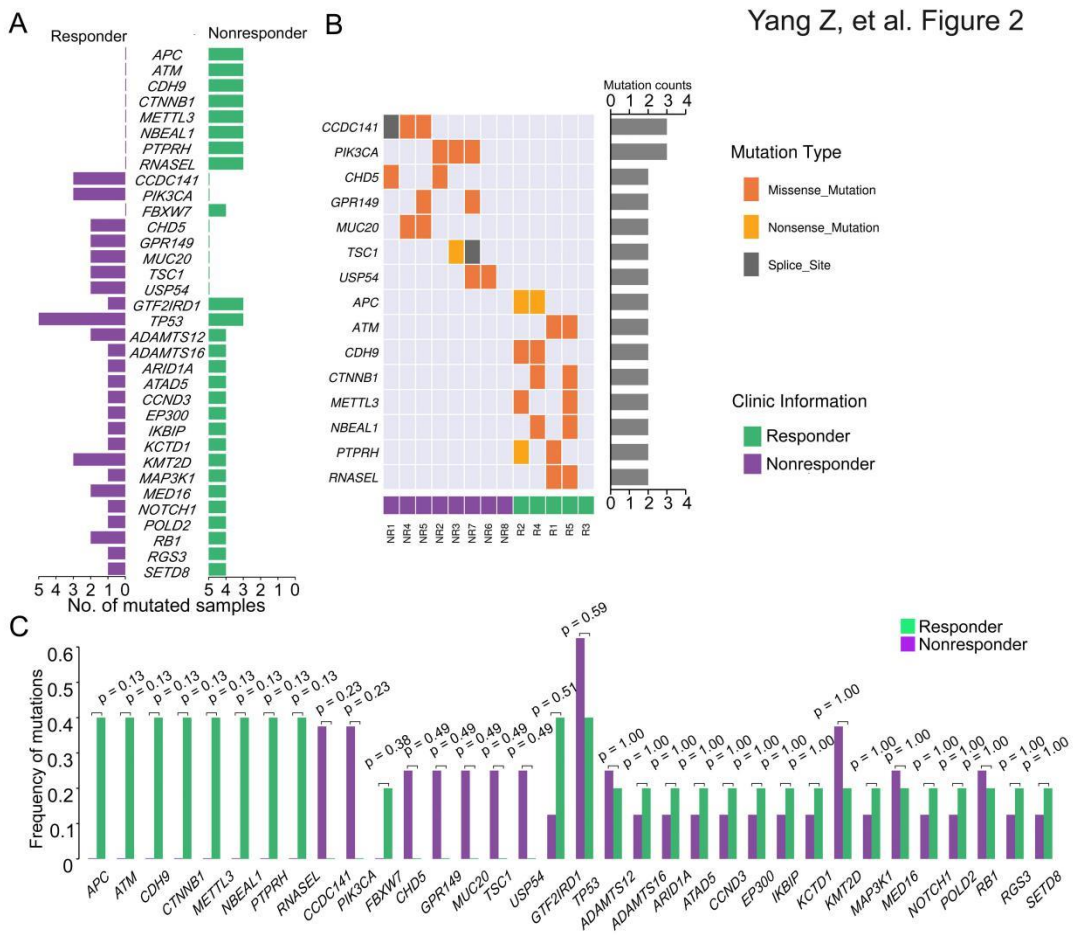
The clinical characteristics including sex, age, **grade**, follow-up time, lymph node metastasis (pN), carcinoma in situ (pCIS) and lymph-vascular invasion (LVI) showed no significant differences between responders and nonresponders at baseline (Table 1). According to TCGA transcriptional subtypes of BC, all the samples were divided into Luminal subtypes (n = 26) and Basal subtypes (n = 7). The Luminal subtypes or Basal

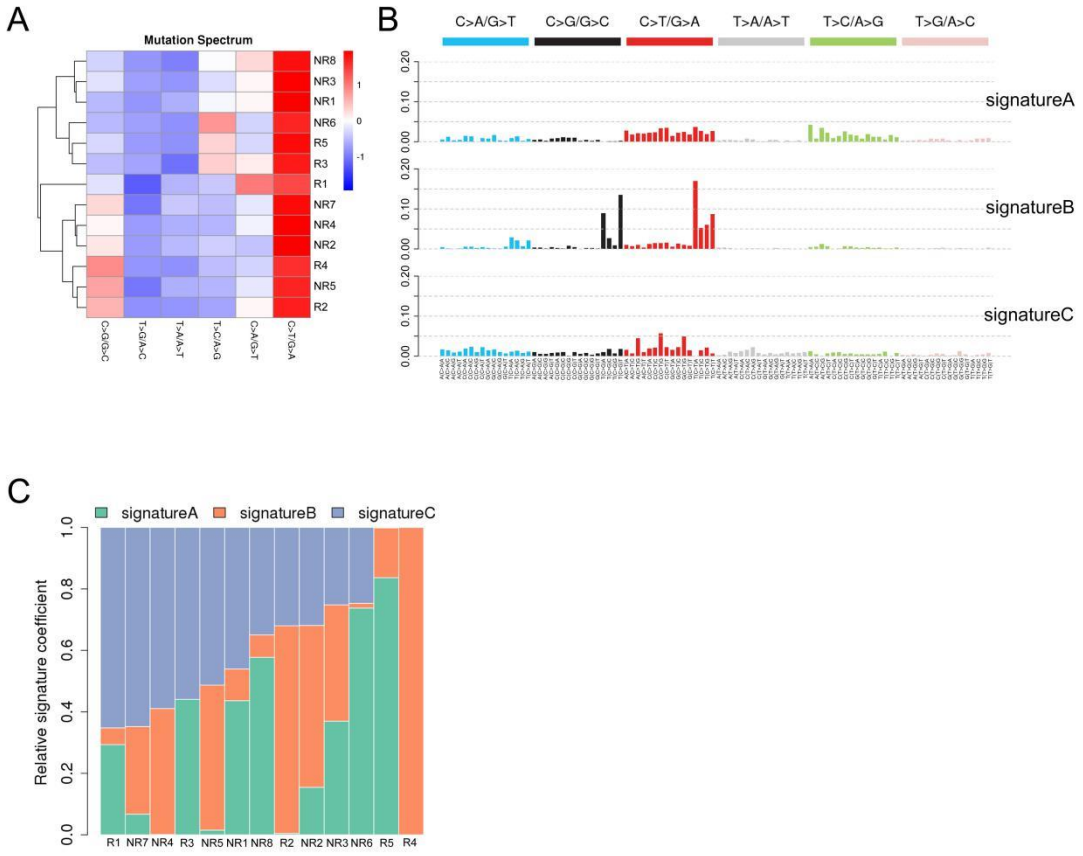
subtypes was not associated with response to NAC (Table 1, p = 0.687).

However, overall survival (OS) and stage (pT) was correlated with non-response (Table 1). (page 6, lines 112-119)

Question 8: Increase font size in Figure 2 panels A and C and Supplementary Figure 2 panel B.

Response: As suggested, font size in Figure 2 panels A and C and Supplementary Figure 2 panel B had been revised.





Supplementary Figure 2. Spectrum of somatic point mutations identified in the 13 muscle-invasive bladder cancer samples. A. A mutation spectrum heatmap of 13 muscle-invasive bladder cancer samples. B.Three mutation signatures identified in the 13 muscle-invasive bladder cancer samples. C. The contributions of mutation signature A-C in each of 13 muscle-invasive bladder cancer samples.

Question 9: The text could use a revision. The mistakes didn't detract from understanding the message, but there are frequent language inaccuracies in the text.

Response: As your advice, we revised the manuscript and improved the language to the best of our ability.

For example, the sentence “NMIBC patient has overall favourable survival rate but high recurrence rate” was changed to “NMIBC patient has a favourable overall survival rate but a high recurrence rate”. (page 4, lines 64-65)

The sentence “MIBC patients have relatively lower five-year survival rate and lesser favourable prognosis” was changed to “MIBC patient has a relatively lower five-year survival rate and a worse prognosis”. (page 4, lines 67-68)

Reviewer #2:

Question 1: There are no page numbers in the PDF, so I will give page numbers that are displayed in the PDF viewer that I'm using.

Response: Thank you for your advice. In order to find the revision, we added the page numbers and lines in the revised manuscript.

Question 2: Discovery (D) cohort: 7/20 patients excluded due to technical failures in sequencing, so n=13, 5 responders and 8 NR. Validation (V) cohort, Sanger sequencing, 16 path responders, 4 NR. The D and V cohorts are relatively small, and there are large differences between the cohorts in WES failures, and in the fraction of responders. Together, these factors make comparisons with other cohorts more important.

-Surprisingly, the comparisons offered are unsatisfying. For example: Page 7, line 1ff: mutated gene frequencies were compared with TCGA 2017, but to my understanding response to treatment was not a parameter in the TCGA work.

-A second example: page 7 line 43ff: the current cBioPortal lists 12 'Bladder cancer' datasets, but the manuscript does not indicate which of these datasets was compared.

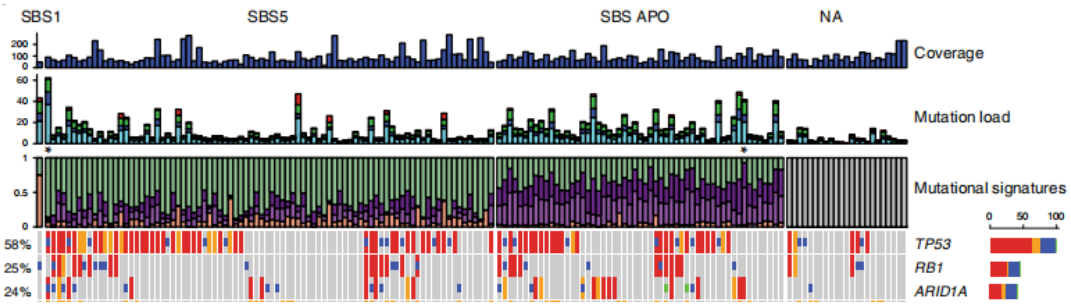
Response: In this study, 40 patients were recruited at the Renji Hospital, School of Medicine, Shanghai Jiaotong University from 2016 to 2019.The patients were divided into discovery and validation cohorts. Each cohort consists of 20 patients. In discovery cohort, seven out of 20 patients were excluded from this study due to technical failures such as DNA extraction, library preparation and exome sequencing. Five patients showed pathological responses while eight patients showed no response. In validation cohort, 16 patients showed pathological response and four patients showed no response. We tried to supplement more samples to both cohorts, but there were no more appropriate samples in Renji Hospital for this study.

As suggested, we pay more attention to the comparison of our findings to the already reported results. Firstly, some of the key genes that have been previously reported as predictive biomarkers of chemotherapy response in bladder cancer, such as DNA damage repair (DDR) genes (*ERCC2*, *ATM*, *RB1*, and *FANCC*), *FGFR3*, *ERBB2* and *BRCA2*. In this

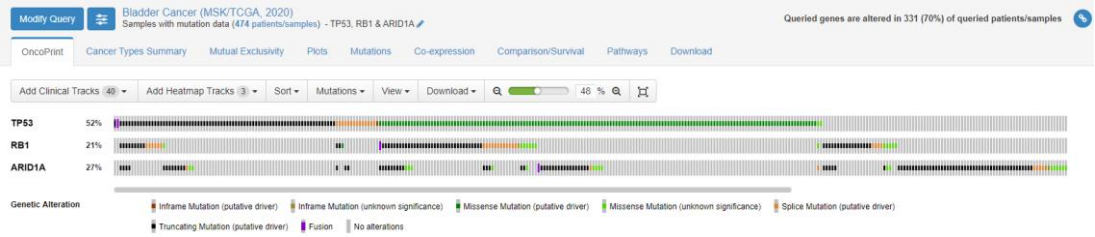
study, *ATM* mutated in 2/21 responders and 0/12 non-responders (Table 1, p = 0.27), *RB1* mutated in 1/5 responders and 2/8 non-responders (Table 1, p = 0.83), and *FANCC* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41). However, the alteration of *BRCA2* was not detected in this study. Furthermore, *FGFR3* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41), *ERBB2* mutated in 0/5 responders and 1/8 non-responders (Table 1, p = 0.41). *ERCC2* mutated in 1/5 responders and 1/8 non-responders (Table 1, p = 0.72). The differences in races, treatment methods and sample sizes might account for this inconsistency. Taken together, the exclusive somatic mutations in the NAC responders and nonresponders would be further examined in the validation cohort. (pages 7-8, lines 159-171).

In the discovery corhort of this study, *TP53* altered in 7 out of 13 samples (54%), *RB1* altered in 3 out of 13 samples (20%), and *ARID1A* mutated in 2 out of 13 samples (15%). In the study of Taber et al. (PMID: 32978382), *TP53*, *RB1* and *ARID1A* mutated with a frequency of 58%, 25%, and 24%, respectively. In TCGA cohort, *TP53*, *RB1* and *ARID1A* mutated with a frequency of 52%, 21%, and 27%, respectively.

Taber et al



TCGA



Taken together, the mutation frequencies of the genes from this study, the study of Taber et al. (PMID: 32978382) and TCGA are similar. The exclusively altered genes in the response group or non-response group displayed elevated mutation frequencies in the response group or non-response group specifically. Furthermore, the study of Groenendijk FH, et al. (PMID: 25636205) and our previous study (PMID: 29941343) also applied the similar comparison to distinguish the biomarkers predicting the response to NAC in MIBC. Thus, the conclusion of this study is truthful.

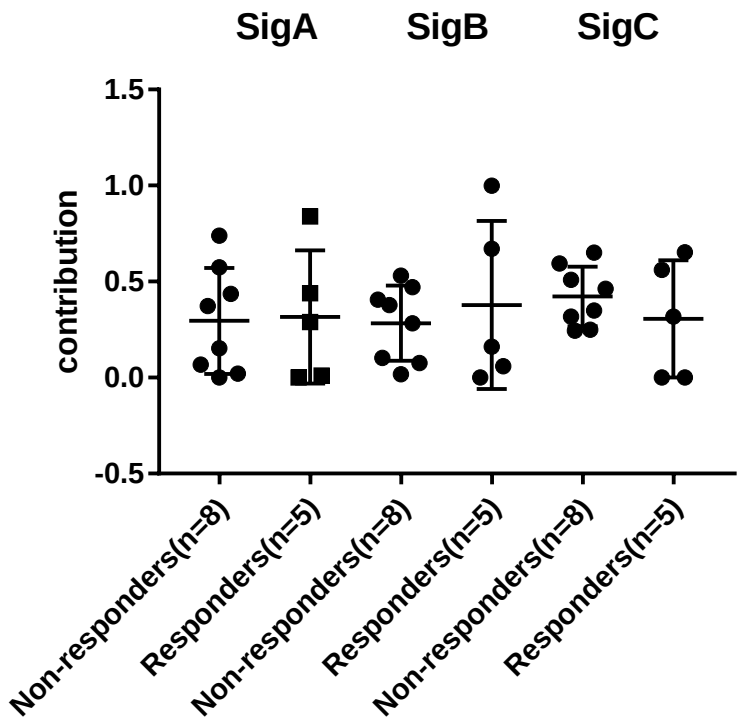
Additionally, in the study of Van Allen et al. (PMID: 25096233), *METTL3* exclusively altered in the responder group (2/25) and *CCDC141* exclusively mutated in the nonresponder group (1/25) (Table 2). However, *PTPRH* altered in both the responder group (1/25) and the nonresponder group (1/25) and no somatic mutations were detected in *CDH9* gene (Table 2). Unfortunately, there were no significant differences between those two groups due to the small sample size. We also tried to compare our result to other studies, including Elizabeth R Plimack et al. (PMID: 26238431), Floris H Groenendijk et al. (PMID: 25636205) and Evanguelos Xylinas et al. (PMID: 27598218). However, we found no records of *CCDC141*, *CDH9*, *METTL3* and *PTPRH* in their mutational results. Taken together, these results suggested that *CDH9*, *METTL3* and *PTPRH* somatic mutations were probably associated with the NAC response, while *CCDC141* mutation was probably associated with the resistance in NAC. (page 9, lines 205-213)

-All the 12 'Bladder cancer' datasets were applied to analyze the OS and DFS between the patients acquiring wild-type *METTL3* and mutated *METTL3*.

Question 3: Page 5, line 21: I do not see a method description of how mutational signatures were identified. I do not see that the reported signatures were compared to previous work.

Response: In this study, we set out to extract the mutation signature characterizing the mutational processes in the discovery cohort as described before (PMID: 22608084). In briefly, all of the somatic SNV detected in 13 patients were included to calculate the fraction of mutations at each of the 96 mutated trinucleotides. Nonnegative matrix factorization (NMF) was employed to extract biologically meaningful mutational signatures which were displayed by a different profile of the 96 potential trinucleotide mutations. Evaluation of NMF decompositions suggested that three mutational signatures was superior, given to the marginal efficiency of the fourth signature. Also, the relative contributions of the three signatures to each case were estimated. (page 17, lines 452-459)

The C->T/G->A mutation dominated the mutation spectrum in 13 MIBC samples (Supplementary Fig. 2A). And three major mutational signatures (A, B and C) were identified in 13 MIBC samples (Supplementary Fig. 2B and 2C, Supplementary Table 7). Refer to Signatures of Mutational Processes in Human Cancer (https://cancer.sanger.ac.uk/cosmic/signatures). The three signatures A, B and C were similar to Single Base Substitution (SBS) Signature 5, SBS Signature 2 and SBS Signature 6, respectively (Supplementary Table 7). Specifically, the contribution of each signature was calculated for each group, and none signature was significantly enriched in nonresponders or responders (Supplementary Table 8). (page 6, lines 132-140)



Question 4: Page 5, line 23: "twenty key mutated genes". Typically 'significantly mutated genes' (SMGs) would be reported. Were the 20 genes SMGs?

Response: In total, *TP53*, *MED16*, *DRC7*, *CEND1*, *ATAD5*, *SETD8* and *PIK3CA* significantly mutated genes (SMGs, Supplementary Table 6) identified in above 13 MIBC samples and 13 key genes associated to the tumorigenesis of bladder cancer were illustrated in a heat map (Fig. 1B). (page6, lines 127-131)

Question 5: Page 14, lines 40ff: the sentence repeats "no high quality variant-supporting reads". This should be corrected.

Response: Thank you very much for your correction, the sentence “while no high quality variant-supporting reads in the tumors” was deleted. (page 16, lines 426)

Question 6: Page 14, line 48: "The preliminary of somatic indels"? It's unclear what this is trying to say. It should be rewritten.

Response: As suggested, the sentence “The preliminary list of somatic indels was called out by GATK based on the local realignment results.” has been revised to “Tumor-specific

somatic mutations were detected by paired blood samples from the patients. Germline mutations were identified and filtered by WES data sequenced by patient's blood." (page 16, lines 429-431)

Question 7: Page 14, line 49: "After that, germline variants could be effectively removed." Methods state that samples were tumour and matched peripheral blood. Would the authors state more clearly how germline variants were identified.

-The samples were presumably frozen (i.e. not FFPE), but I do not see this stated.

Response: Tumor-specific somatic mutations were detected by paired blood samples from the patients. Germline mutations were identified and filtered by WES data sequenced by patient's blood. (page 16, lines 429-431)

-As suggested, the Sample collection and preparation section has been revised as follows: "And then tumor tissues and peripheral blood cells were frozen at liquid nitrogen and stored at ultralow temperature freezer." (page 15, lines 378-379)

Question 8: Page 14, line 60: "indels represented by only one DNA strand" How were such indels identified, i.e. distinguished from indels present on both strands? Why should these be ignored?

Response: DNA was composed of two strands. When the sequencing reads remapped to the reference, the reads were labeled by "+" and "-", which referred to the two strands. If the reads supporting mutations were all "+" or "-", we tended to regard it as a false calling. This filter concerning strand bias was adopted by numerous previously researches (PMID: 22608084 and PMID: 26215952).

Question 9: Page 14 line 61: ignore "substitutions located 30 bp around predicted indels". Why should these mutation calls be ignored?

Response: Cluster of substitutions and indel is associated with repeat sequence. The accuracy of mutation calling was unsatisfied if the predicted indels were closed by a substitution.

Referred as “Proximal Gap”, mutation callers always exercised great caution in dealing with this situation (PMID: 23396013).

Question 10: Page 15 lines 1: "false positive associated pseudo gene issues" It's unclear what this is trying to say. Please rewrite this to be clearer.

Response: Sorry about it, we changed the sentence to “To filter out the false positive, such as repeat sequences, simulated reads (80 bp in length) containing the potential mutations were generated and aligned to the reference genome.” (page 17, line 439-441)

Question 11: Page 15 line 39: DAVID, "specifying $p < 0.05$". Was a p value used that was corrected for multiple hypothesis testing?

Response: As suggested, the p value was corrected for multiple hypothesis testing. However, the corrected p values are ≥ 0.05. Thus, the Pathway enrichment section has been deleted from the manuscript. Thank you very much for you advice.

2nd Editorial decision
14-Apr-2021

Ref.: Ms. No. JCTRes-D-20-00137R1
Alteration of METTL3 Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by May 14, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

I apologize for the delay in rendering a decision on your manuscript. The delay was caused by us having to wait on one of the reviewers, who failed to comply with multiple requests from us to submit a re-appraisal of your manuscript. Accordingly, we decided to take your manuscript out of peer review and perform a review ourselves.

We find that your revision and rebuttal satisfy most of the concerns that had been raised by the reviewers. Your manuscript does not have to be re-reviewed again.

However, the manuscript text does not conform to our requirements regarding academic level English, as was pointed out by both reviewers. We kindly ask you to involve a native speaker to correct the English, or engage a service provider to proofread the manuscript. If you cannot manage either, please contact the editor (m.heger@jctres.com) so that we can try to help you find linguistic support for a fee.

Thank you and kindest regards,

Michal Heger
Editor

Authors' response

Response to Editor's and Reviewers' Comments

Dear Editor-in-Chief Dr. Michal Heger:

Attached please find the revised version of our manuscript entitled "Alteration of *METTL3* Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer" (No. JCTRes-D-20-00137). We appreciated very much for those valuable comments and helpful suggestions from you and the reviewers, which have guided us to significantly improve the quality of our manuscript. We have thoroughly revised the manuscript accordingly with check changes.

Responses to Editor' comments

Question 1: The manuscript text does not conform to our requirements regarding academic level English, as was pointed out by both reviewers. We kindly ask you to involve a native speaker to correct the English, or engage a service provider to proofread the manuscript.

Response: Thank you very much for those valuable comments and from you and the reviewers and we have thoroughly revised the manuscript accordingly with check changes.

3rd Editorial decision
27-Apr-2021

Ref.: Ms. No. JCTRes-D-20-00137R2
Alteration of METTL3 Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by May 27, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

Thank you for resubmitting your manuscript to JCTR and for making an effort to improve the linguistics of your paper.

Unfortunately, the language is still not up to par and I kindly ask you to involve a professional service or a native speaker in helping you to upgrade the manuscript text and eliminate errors.

For example, syntax errors such as "used THE whole-exome sequencing" and "occurred in NAC RESPONDER" in the abstract alone indicate that your paper was not reviewed by a native speaker, as such mistakes would not have been left in the manuscript.

We have in-house editors who could help with the language for a fee if you cannot find the appropriate service or assistance. Just let me know, please, in case you want to use our services (m.heger@jctres.com).

This situation is very unfortunate, as I think your paper is important and clinically relevant and I would like to proceed with publication.

Thank you for ensuring that the manuscript in the next round is sound and in tip top shape linguistically.

Kindest regards,

Michal Heger
Editor

Authors' response

Response to Editor's and Reviewers' Comments

Dear Editor-in-Chief Dr. Michal Heger:

Attached please find the revised version of our manuscript entitled "Alteration of *METTL3* Predicts Response To Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer" (No. JCTRes-D-20-00137). We appreciated very much for those valuable comments and helpful suggestions from you and the reviewers, which have guided us to significantly improve the quality of our manuscript. We have thoroughly revised the manuscript accordingly with check changes.

Responses to Editor' comments

Question 1: The language is still not up to par and I kindly ask you to involve a professional service or a native speaker in helping you to upgrade the manuscript text and eliminate errors.

For example, syntax errors such as "used THE whole-exome sequencing" and "occurred in NAC RESPONDER" in the abstract alone indicate that your paper was not reviewed by a native speaker, as such mistakes would not have been left in the manuscript.

Response: As suggested, a professional service had been applied for this manuscript. We hope it will satisfy the requirement of the journal.

4th Editorial decision
10-May-2021

Ref.: Ms. No. JCTRes-D-20-00137R3
Mutations of METTL3 Predict Response to Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: