Author’s response to reviews

Title: Unbiased data mining identifies cell cycle transcripts that predict non-indolent Gleason score 7 prostate cancer

Authors:

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Author’s response to reviews:

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Kenneth Iczkowski

BMC Urology

https://bmcurol.biomedcentral.com/

Dear Dr. Iczkowski,

Attached, please find a revised manuscript “BURO-D-18-00143 Unbiased data mining identifies cell cycle transcripts that predict non-indolent Gleason score 7 prostate cancer, by Wendy L. Johnston, Charles N. Catton, and Carol J. Swallow”.

We wish to thank you and Drs. Singh and Kravtsov for your time and helpful suggestions for editing our manuscript. We have made the suggested revisions, including stratifying the Gleason 7 data into Gleason 3+4=7 and Gleason 4+3=7. As well, we have included data on Gleason 6 tumours. While stratifying the Gleason 7 data, we detected a transposition error, which has been corrected (resulting in updated Table 2 and Figures 2, 3). Detailed responses to the editor’s and reviewers’ comments are provided below.

Thank you, and looking forward to your reply.

Sincerely,
Wendy L. Johnston, Charles N. Catton and Carol J. Swallow

Detailed Response to Editor’s and Reviewer’s questions:

Editor’s comments:

Reviewer 1 asks:

1) Whether the 13 transcripts are alternately regulated in low-risk, as well as in intermediate risk. The authors have defined low risk as stage pT2a. Since pT2a, pT2b, and pT2c ar all merged, the authors should instead study Gleason 3+4=7 versus 4+3=7 to compare BCR outcomes for lower-risk Gleason 7 versus higher-risk Gleason 7. This should be feasible, since the authors state that Gleason score was available for all cases.

Response A: We have included information on Gleason 3+4=7 and 4+3=7 in the Background (lines 79-81) and reanalyzed the data as suggested using Gleason 3+4=7 and Gleason 4+3+7, instead of tumour stage (Methods (lines 155-156)). Our results (new Tables 3 and S6) show that the transcripts are especially predictive of poor outcome in Gleason 3+4=7, a finding that has been discussed in the revised manuscript (lines 205-208, 211-216). We have also removed the T category data from Figure 2 (“Figure 2C” in the original submission, Additional File 2, as well as the original Table 3, which examined multivariable logistic regression using transcript abundance and tumour T category).

2) Perform MVA including PSA levels and transcript abundance as variables. Also, 3+4 versus 4+3 should be a variable.

Response B: PSA was not used in the multivariable analysis for two reasons: i) Pre-operative PSA was only available for 88/156 patients, and of these 88, only 4 experienced BCR, which was not a large enough number of events to test PSA as a second independent variable in the multivariable analysis. This information was included in the original submission, (lines 166-168), and is retained in the revised submission,( lines 158-160). ii) Within the group of patients for whom pre-operative PSA was available, the ROC-AUC was not statistically significant (P=.087; Supplemental Figure S1 in the original and revised submissions). Regarding: 3+4 and 4+3, please see response A, above.
3) The reviewer asks about drugs administered, age, and race. If the authors want to tackle this they may, but I consider it optional since the authors may not have access to drug therapy, age, or race data.

Response C: As it was considered optional by the editor, we did not address drugs administered, age, or race.

4) Do the patients develop castrate-resistant prostate cancer? If the authors have access to CRPC status, I consider it optional to answer that question. BCR is good enough as an end point.

Response D: We used BCR as the end point since the TCGA follow-up time was short and did not include longer-term data on castrate-resistant prostate cancer.

5) Yes, reference format needs to be fixed.

Response E: The reference format has been changed to the suggested Vancouver format.

Reviewer 2 asks:

Makes the point about stage and grade classification used in the study not being up to date. The authors used the AJCC 7th edition system of pT2b or pT2c as intermediate risk, and pT2a as low risk. These have been collapsed together in the 8th edition of AJCC classification and should not be analyzed separately. [https://cancerstaging.org/About/news/Pages/Prostate-cancer--major-changes-in-the-American-Joint-Committee-on-Cancer-eighth-edition-cancer-staging-manual%E2%80%8B.aspx](https://cancerstaging.org/About/news/Pages/Prostate-cancer--major-changes-in-the-American-Joint-Committee-on-Cancer-eighth-edition-cancer-staging-manual%E2%80%8B.aspx). Also, the grade grouping system with 5 different grade groups should be used to substratify Grade Group 2 (3+4=7) versus Grade Group 3 (4+3=7) since these have known different outcomes. As noted above, the multivariate analysis should use Grade Group 2 versus Grade Group 3 as one of the variables. Here is information on the grade group system: [http://pathology.jhu.edu/ProstateCancer/NewGradingSystem.pdf](http://pathology.jhu.edu/ProstateCancer/NewGradingSystem.pdf)

Response F: As outlined in Response A, we have stratified the Gleason 7 tumours into 3+4 and 4+3 and have redone the analysis accordingly, instead of using tumour pT classification.

Reviewer comments:

Amrita Singh (Reviewer 1): Johnston and colleagues provide interesting data in this study identifying at least 13 transcripts in Gleason score 7 patients that may help predict risk of
developing advanced disease. The authors use different data visualization and analysis tools to interrogate multiple publicly available prostate cancer datasets. A test set of biomarkers was created based on set criteria and then validated. 11 transcripts were identified that are upregulated and may be associated with disease recurrence and advancement to castrate resistant prostate cancer. Overall, this study attempts to answer an important question in the field of prostate cancer which is to identify biomarkers that are predictive of outcome and help in clinical decision making for patients who fall in the intermediate risk group. The manuscript is well written and the data are well presented.

Comments-

1) Is the upregulation of 11 transcripts unique to Gleason score 7 patients or are these genes differentially regulated in low risk patients also? Compare transcript abundance of the 13 transcripts of interest between Gleason score for low risk and intermediate risk.

Response G: As suggested, we have extended the analysis to include low risk Gleason 6 patients (Supplemental Table S5, lines 208-211) and have added a table showing the relative changes in abundance of transcripts in different Gleason scores (Supplemental Table S7 in the revised submission)

2) Perform multivariate regression analysis using PSA levels and transcript abundance as variables.

Please see Response B, above.

3) Is there a correlation between the effect of treatment drugs administered, age related, race related differences in different Gleason score 7 patients for therapy and expression of 11 transcripts that are up regulated?

Please see Response C, above.

4) Since the 11 cell cycle and mitosis genes are associated with HES6-dependent E2F1 transcription factor mediated CRPC,(in this study E2F2). Do the patients with Gleason score 7 tested in this study develop CRPC? This information will support robustness of these 11 transcripts to be used as biomarker for prediction of outcome.

Please see Response D, above.
Minor comments-

1) The references need to be cited in the recommended style of journal.

Please see Response E, above.

Oleksandr Kravtsov, MD (Reviewer 2): Please, use the latest 8th edition of AJCC Cancer Staging Manual as a reference (rather than 7th). pT2 is no longer subclassified by extent of involvement or laterality 8th edition of AJCC, and that affects the criteria used in this study for intermediate risk stratification. Gleason grade groups are also used for risk stratification in AJCC 8th ed.

Please see Response F, above.

The authors performed a study using innovative data mining approach and got additional data on prostate cancer prognostic markers. Although more data and prospective studies are needed to make a significant clinical impact this study would be valuable addition to prostate cancer knowledge base.