Author’s response to reviews

Title: Machine learning enables detection of early-stage colorectal cancer by whole-genome sequencing of plasma cell-free DNA

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**Author’s response to reviews:**

June 30, 2019

Dear Dr. Rice,

Thank you for sending the additional reviewer’s assessment of our manuscript. We are very interested in having a revised version of our manuscript published in BMC Cancer. To that end, please find attached our revised manuscript, “Machine learning enables detection of early-stage colorectal cancer by whole-genome sequencing of plasma cell-free DNA”, as well as a point-by-point response to the statistical reviewer’s comments.

We thank you and the reviewers again for your critical review of our manuscript, which we believe is much improved as a result. We hope you will now find it suitable for publication in BMC Cancer.

Sincerely,

Girish Putcha, MD, PhD
Chief Medical Officer
Freenome
Response to Reviewers' comments on manuscript, “Machine learning enables detection of early-stage colorectal cancer by whole-genome sequencing of plasma cell-free DNA” by Wan et al.:

Research Square (Reviewer 3): "STATISTICAL REVIEWER ASSESSMENT:

REQUESTED REVISIONS:

1. Since hyperparameter search is used, the data that the reported final performance is computed on (e.g. test data) must not be overlapping with the validation data used for hyperparameter search. As the authors did not describe this in detail, I assume typical cross-validation scheme was used. Then modifications have to be made to make the reported performance unbiased. As this manuscript has a major focus on cross-validation schemes, this issue should be addressed rigorously.

We apologize for any lack of clarity. The hyperparameter grid search over the training data of each fold is now detailed in the Supplementary Material (Classification, Page 4, Line 89 and 90). Importantly, hyperparameters were not selected from the results of the test set, and we have updated the Methods section (Page 9, lines 191-193) in the manuscript to reflect this.

Although cross-validation (CV) is a widely used approach for assessment of classification performance, the use of an independent test set is ultimately needed to evaluate the generalizability of CV results. However, like other retrospective proof-of-concept studies of this scale (e.g., Cohen et al., Science, 2018; Cristiano et al., Nature, 2019), an independent test set was not available.

The reviewer is correct in that a typical CV approach was used. In our previous versions, this description of our cross-validation process was included in the Supplemental Methods section; we have now revised the Methods section in the main manuscript to include a more detailed description of our cross-validation (Methods, page 9 line 199-200).

2. The other issue I discussed above ("simultaneously address multiple confounders") should also be discussed and ideally addressed."

Stated above: A major limitation of the study design, however, is that the authors' approach cannot (or it is challenging to) simultaneously address multiple confounders. In particular dataset, it appears ok because only one factor - "institution" - seems to be a confounder of high effect). But this may not be the case in other datasets, limiting the applicability of this method. Moreover, even in the authors' dataset, other confounders, despite with relatively lower effects, are still predictive of tumor-control status, meaning that all reported performance scores are not clean of confounding effects.
We apologize again for any confusion we caused and wholeheartedly acknowledge that this is a limitation of our manuscript. However, unlike the other retrospective studies cited above, we attempted with ordered k-batch and balanced k-batch to simultaneously address the confounding impact of batch and processing date and of batch and institution, respectively. In the revised manuscript, we have also improved our analysis of confounding by age (Figure 3b).

We are unaware of any methods that exist to completely remove the impact of all confounding variables on clinical performance for our particular application. It is important to point out that we are not proposing methods in this manuscript to overcome these confounders; we are providing an assessment of certain confounders. Our current manuscript describes methods for assessing the impact of confounding variables in our models; however, methods for simultaneously correcting for multiple confounders is an area of continued research for us. To better communicate this point, we have clarified the language in the manuscript (Results, page 11, line 242-247). In addition to such work, we are currently enrolling prospective studies to improve and evaluate the performance of our models in completely independent, fully intended use populations of patients. We have updated language in the discussion to address this (Discussion, page 17 and 18, lines 388 to 394).