Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-445

Reviewer A

Comment 1: Authors mentioned that this study quickly identified effective therapeutic targets for precision treatment of HR+/HER2- breast cancer. It would be better if authors could specify which effective therapeutic targets have been found and their possible impact on clinical decision making.
Reply 1: Thank you for your suggestions. We have found that some therapeutic targets can be well predicted including TP53 mutation, GATA3 mutation, the G2-M checkpoint pathway activation and PI3K/AKT/mTOR signaling pathway activation. We described the significance of these markers in the discussion of the revised manuscript (see Pages 18-19, lines 434-451). Thank you!
Changes in the text: We have modified our text as advised (see Pages 18-19, lines 434-451)".

Comment 2: It would be better to validate the accuracy of the model using external data.
Reply 2: Thank you for your suggestions. The Cancer Genome Atlas (TCGA) is a public database with whole slide images. However, the definition of ER, PR and HER2 expression in TCGA is different from the current definition. Therefore, it is hard to accurately identify HR+/HER2- breast cancer in TCGA. We have explained the lack of external validation of our study in the revised manuscript (see Page 20, lines 482-485). Thank you!
Changes in the text: We have modified our text as advised (see Page 20, lines 482-485)".

Reviewer B

Comment 1: There is no information of the included patients about HR or HER2 expression in the abstract.
Reply 1: Thank you for your comments. We have added information about the HR and HER2 expression of the included patients in the abstract of the revised manuscript (see Page 2, lines 35-37 and line 44).
Changes in the text: We have modified our text as advised (see Page 2, lines 35-37 and line 44)".

Comment 2: The deep-learning-based workflow in conclusion is not mentioned in Methods or Results of abstract.
Reply 2: Thank you for your comments. The deep learning-based workflow included a neural network model for tissue type segmentation and another one for predicting different therapeutic targets based on specific tissue type image tiles. We have added the information to the Methods and Results section of the abstract in the revised manuscript (see Page 2, lines 38-40 and 44-47). Thank you!
Changes in the text: We have modified our text as advised (see Page 2, lines 38-40 and

lines 44-47)".

Comment 3: The method, Deep Learning should be expressed more clearly.
Reply 3: Thank you for your suggestions. We have added some details of the deep learning algorithm that we used in the Methods section in our revised section.
Changes in the text: We have modified our text as advised (see page 5, lines 118-123).

Reviewer C

Comment 1: It does not explain CNN network architecture, how many layers of convolution and the structure of each layer?
Reply 1: Thank you for your good question. We used the ResNet-18 as our CNN network architecture, which is a commonly used residual neural network. We have added the reference of ResNet-18 architecture in our revised manuscript.
Changes in the text: We have modified our text as advised (see Page 8, lines 176-178; Page 10, lines 240-241)".

Comment 2: What is the loss function used by CNN? it does mention in the paper.
Reply 2: We used cross-entropy loss as the loss function. We have added this information in the revised manuscript (see Page 8, line 179; Page 10, line 242).
Changes in the text: We have modified our text as advised (see Page 8, line 179; Page 10, line 242)". Thank you!

Comment 3: How to explain the mutation of GATA3? In most breast carcinoma, GATA3 is positive in IHC while it is used to distinguish tumor originated from breast or other organ in clinical diagnosis.
Reply 3: Thank you very much for your kind suggestions. We have added the clinical relevance of GATA3 mutation in the discussion in our revised manuscript (see Page 18, lines 439-440). Thank you!
Changes in the text: We have modified our text as advised (see Page 18, lines 439-440)". Thank you!

Reviewer D

Comment 1: I am wondering how the tiles with limited tissues were handled. Tissue samples are generally irregularly shaped and many regions with empty tissue cannot be covered by the framing tool in ImageScope software.
Reply 1: The white background part in the ROI area were filtered out during MATLAB image tiling. Image tiles with limited tissues (defined as more than half of the pixels within the tile were > 210) were also discarded (1). Thank you!
Changes in the text: We have modified our text as advised (see Page 7, lines 164-166)". Thank you!

Comment 2: What are the criteria for ROI selection? How many subregions were selected per patient?
Reply 2: We used the rectangle tool of ImageScope software to generate one or two rectangles ROIs that included the vast majority of the invasive breast cancer areas and excluded majority of the dragged tissue and background areas. We have added the information in our revised manuscript.
Changes in the text: We have modified our text as advised (see Page 7, 157-160)". Thank you!

Comment 3: The authors failed to describe the architecture and parameters of the deep-learning model, which should be made very clear in a methodology paper.
Reply 3: Thank you very much for your kind suggestions. We have described the architecture and parameters of the deep-learning models used in our study in our revised manuscript (see Page 8, lines 176-181; Page 10, lines 240-243). Thank you!
Changes in the text: We have modified our text as advised (see Page 8, lines 176-181; Page 10, lines 240-243)". Thank you!

Comment 4: Why use three-fold cross-validation instead of five-fold?
Reply 4: We referred to two studies that also predicted tumor multi-omics features. We have added these two reference in our revised manuscript(1, 2).
Changes in the text: We have modified our text as advised (see Page 12, lines 290). Thank you!

Comment 5: I appreciate the authors attempted to interpret the biological implications of good versus poor predictions based on morphological features. However, this part is lack of depth as all analyses were visually performed without any computational validation. I would recommend the authors perform cell segmentation and phenotyping on those tiles and statistically compare the cellular heterotypic, mitosis distribution, etc.
Reply 5: Thank you for your good suggestions. In this study, we focused more on local tissue morphological patterns rather than on individual cell phenotypes in the prediction of the clinicopathological features and multi-omics features. We will consider using your approach to further illustrate the association between molecular features and image features in future work.
Changes in the text: Page 20, lines 481-482

Comment 6: What are the criteria when conducting the primary quality review? What if there are disagreements between the two pathologists?
Reply 6: We have added the quality review criteria in our revised manuscript (see Page 6-7, lines 147-153).
Changes in the text: We have modified our text as advised (see Page 6-7, lines 147-153)". Thank you!

Comment 7: The authors mentioned the name of the six cancer-related pathways almost at the end of the manuscript (line 346), I would recommend make it mentioned in the

earlier part.

Reply 7: We added the description of the six cancer-related pathways to the method section in our revised manuscript (see Page 8-9, lines 199-202).

Changes in the text: We have modified our text as advised (see Page 8-9, lines 199-202)". Thank you!

References

1. Zhao S, Yan C-Y, Lv H, et al. Deep learning framework for comprehensive molecular and prognostic stratifications of triple-negative breast cancer. Fundamental Research. 2022.

2. Kather JN, Heij LR, Grabsch HI, et al. Pan-cancer image-based detection of clinically actionable genetic alterations. Nat Cancer. 2020;1(8):789-99.