Author's response to reviews

Title: The Potential Biomarkers in Predicting Pathologic Response of Breast Cancer to Three Different Chemotherapy Regimens

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Author’s response to reviews: see over
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Andrea Bucceri, Ph.D.
Scientific Editor
BMC-series Journals
BioMed Central
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London, WC1X 8HL

Dear Dr. Andrea Bucceri:

Thank you very much for providing us the opportunity to revise the manuscript entitled “The Potential Biomarkers in Predicting Pathologic Response of Breast Cancer to Three Different Chemotherapy Regimens” (#1179592495246112). According to your instruction and the reviewers’ comments, we have carefully revised this manuscript (highlighted in red). We are now resubmitting the manuscript for your examination.

Please see next page in which we describe in details of our modifications or corrections of the content of the manuscript point by point based on reviewers’ comments and suggestions. We hope that these changes would make this revised manuscript acceptable.

Thank you for your time and consideration. Please let me know if you have any questions or need further information.

Sincerely,

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Responses to Reviewer 1 (Dr. Alfredo Berruti):

1. Thanks for the reviewer’s comment. This clinical study was different from many other clinical studies on cancer therapeutics because we need to collect human tissues and perform pathological/histological examinations. Thus, it would be difficult for us to have large groups of patients for each chemotherapeutic regimen. However, the results we obtained should provide some useful information or clues for possible roles of these markers on combination chemotherapy, although the number of patients was relatively small compared to other clinical studies. We believe the results are reliable and worth further study.

2. As described in our manuscript and commented by the reviewer, the independent predictive role of ER for response to treatment has also been observed by several other groups. Considering that it might not be necessary to list all those publications, we have selected and cited 5 of them in our manuscript. Our statement that “Previous investigations evaluating the potential correlation between estrogen receptor status and pathologic response to chemotherapy have produced contradictory data” is based on a number of previous reports indicating that ER-positive tumors respond either better (Ref. 21), less well (Ref. 22-27) or the same (Ref. 28-31), when compared with ER-negative tumors. In addition, we have evaluated a panel of biomarkers and found ER is an independent predictive factor for pathologic response (PR) to preoperative chemotherapeutic regimens (PCT) including DEC, VFC and EFC in primary breast tumors, while HER2 is only predictive for DEC regimen. Expression of PgR, Topo-II, P-gp, MRP and GST-pi are not predictive for PR to any PCT regimens investigated.

3. As the reviewer mentioned, proliferative activity is increasingly recognized as an independent predictive factor of chemotherapy efficacy. In fact, Ki-67 was included in the panel of markers in our study. However, due to the small size of biopsy samples, we were not able to get the Ki-67 data of 10 patients enrolled in this study. But the biopsy tissues from remaining 108 patients were successfully evaluated for the Ki-67 expression. Our statistical data indicated that Ki-67 expression was inversely correlated with ER ($p=0.000$). In invariant analysis, high Ki-67 expression was associated with increased pathologic response to preoperative chemotherapy ($p=0.037$), but when we assessed the effect of Ki-67 with Cochran-Mantel-Haenszel chi-square test adjusted for the effect of a stratification variable ER, Ki-67 expression was not associated with increased pathologic response ($p=0.717$). Furthermore, in multivariate analysis, Ki-67 was not an independent variable ($p=0.720$). Based on these data, we believe that the greater response rate observed in ER- tumors was not a result of higher expression of Ki-67 in the breast tumors evaluated in this study. Considering that the data with Ki-67 was evaluated with 108 patients while all other biomarkers were obtained with 118 patients, we did not add the above data in our manuscript.
4. In our study, the positive rate of HER2 was 58.5%, which was greater than the average positive ratio of HER2 in breast tumors. We have re-evaluated the HER2 expression before this revision and got the same positive rate as above. It has been shown that more advanced breast tumors may have relatively higher level of HER2 expression (new Ref. 35). Based on the literature, we think that the high proportion of HER2+ tumors may be due to the advanced clinical stage of breast cancer patients enrolled in this study. We added this explanation in “Discussion” of this revised manuscript (page 11-12).

5. Following the reviewer’s suggestion, we have added the percentage of positive rate of immunohistochemical markers in Table 2 of the revised manuscript.

6. According to the reviewer’s comment, we have deleted this conclusion in both “Abstract” and “Conclusions”, and only mentioned this issue in “Discussion” session.

Response to Reviewer 2 (Dr. Yuval Shaked)

1. In our study, three different chemotherapy regimens were used in breast cancer patients, including DEC (docetaxel+epirubicin+cyclophosphamide), VFC (vinorelbine/vincristine+5-fluorouracil+cyclophosphamide) and EFC (epirubicin+5-fluorouracil+cyclophosphamide). As the reviewer mentioned, the VFC regimen is currently not widely used to treat breast cancer patients. However, vinorelbine (V) in this regimen is an important drug for metastatic breast cancer and is recommended by NCCN clinical practice guidelines. On the other hand, the major purpose of this study was to identify biomarkers that may predict pathologic response of breast cancer to chemotherapy regimens. Our data showed that ER was an independent predictive factor for pathologic response to all the above three chemotherapeutic regimens. Thus, the findings obtained in this study should provide useful information for optimization of chemotherapy for breast cancer patients.

2. Thanks for the reviewer’s kind suggestion. In this study, all the experiments were completed in the pathology laboratory of Sir Run Run Shaw Hospital. We have added this information in the “Methods” session (page 5). Indeed, the variability between laboratories in the assessment of protein expression by using immunohistochemical techniques could be high. We will follow the reviewer’s suggestions in our future randomized studies.

3. Thanks for the reviewer’s reminder. We have added the related information in the “Method” session of the revised manuscript (page 5). In brief, tumor tissues were obtained 2-3 days prior to chemotherapy via core biopsy or during surgery. (performed after two weeks of the last cycle of chemotherapy).

4. Paclitaxel and doxorubicin are widely used chemotherapeutic drugs for breast
cancer patients. Due to the limitation of enrolling enough patients for each chemotherapy regimen, paclitaxel and doxorubicin were not included in this study. However, docetaxel and epirubicin are new generation of taxanes and anthracycline family of chemotherapeutic drugs. Furthermore, our recent studies indicated that ER mediates resistance to paclitaxel in breast cancer cells in vitro (Sui M, et al, Cancer Research, 67:5337-5344, 2007). Moreover, our previous studies have shown that docetaxel and paclitaxel possess similar responses to steroid-mediated drug resistance. Thus, data obtained from this study may provide useful information for clinical use of paclitaxel and doxorubicin. Evaluating pathologic response to these two drugs in breast cancer patients will be considered in our future clinical studies.