Title: Alterations of the genes involved in the PI3K and estrogen-receptor pathways influence outcome in human epidermal growth factor receptor 2-positive and hormone receptor-positive breast cancer patients treated with trastuzumab-containing neoadjuvant chemotherapy

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Author's response to reviews: see over
Reviewer number: 1
Referee's comments to the author(s)
This is a nicely written paper summarizing molecular analyses on tumor tissue from patients with HER2+ breast cancer treated with trastuzumab-based therapy in the neoadjuvant setting. A few points need to be considered:

(1) The entire manuscript would be improved by making more succinct and to the point. The results and discussion sections are far too detailed and long, making it difficult for the reader to understand the main points. The tables are very detailed and so could be used to refer reader to minor details rather than stating every point in the text. For example, page 13 the statement, "There were no differences in the incidence of gain of DEK, FGFR1, CCND1, FOXA1, MYML2, or AIB1 between the two types of tumors," could be removed and the information provided in the table would suffice.

Many thanks for the comments: We followed the advice, and deleted Table 6 and the relevant paragraphs and sentences in the abstract (page 3, lines 19 and 20), results (pages 15 and 16), and discussion (page 19) sections. We also transferred the original Table 4 to the additional Table S2 in the revised manuscript. The sentence in page 13 pointed out by the reviewer and the similar detailed sentence in page 12 were removed. We believe that the revised manuscript has been substantially shortened and become more readable.

(2) The fact that pCR did not predict better RFS is not consistent with multiple large neoadjuvant clinical trials (including meta-analysis presented by Cortazar at San Antonio 2012). This result is likely due to the small sample size in this study and should be acknowledged as such in the Discussion section.

We added a new paragraph discussing the contradictory finding on the relationship between pCR and RFS in the present study in the revised manuscript (page 20). We also added four new references (Ref Nos. 2, 43, 44, and 48) in the background and discussion sections; No. 44 was written by Cortazar and others.

(3) There are several areas in the manuscript where the authors make statements such as "HER2+ patients" or "HR+ patients." These phrases should all be changed to state, "patients with HER2+ tumors..." etc as the patients themselves are not HER2+, the tumors are.
"HER2+ patients" or "HR+ patients" were changed to patients with HER2+ tumors or patients with HR+ tumors throughout the manuscript.

(4) The following statements are overreaching and should be revised as they are not supported by the results in this paper. "Thus, these findings provide biological evidence that supports the concurrent use of trastuzumab, lapatinib, and an anti-hormone agent for HER2+/HR+ patients. Cytotoxic drugs should be also added to obtain a better outcome." This statement is only supported by 2 papers referenced by the authors. In those studies, patients with HR+HER2+ breast cancer treated with lapati/tras still have lower pCR rates than those that are HR neg/HER2+. So adding lapatinib does not entirely reverse resistance to HER2-targeted therapy. In the manuscript being considered, no patients were treated with lapatinib, thus concluding that this work supports use of concurrent tras/lapat is unfounded.

We followed the comment, and rewrote the relevant sentences in the abstract (conclusions), discussion (the last paragraph), and conclusion sections. The revised paragraph in the discussion are “The present study showed that the PI3K and ER pathway genes were specifically altered in HER2+/HR+ tumors of patients who subsequently relapsed after receiving neoadjuvant chemotherapy with trastuzumab. Lapatinib targets the intracellular ATP domain of HER2, preventing self-phosphorylation and subsequent activation of the PI3K and MAPK signal pathways [5]. It may be reasonable to add lapatinib to the present trastuzumab- containing chemotherapy for patients with a HER2+/HR+ tumor to overcome the resistance due to the altered PI3K pathway genes and to obtain a better outcome.” (pages 20 and 21)

Reviewer number: 2

Referee's comments to the author(s)

Takada et al present interesting evidence suggesting that the PI3K and ER pathways affect response to neoadjuvant and adjuvant trastuzumab in patients with HER2+ breast cancer. The authors performed a comprehensive analysis of markers of these pathways in association with pathological response and outcome. Several issues should be addressed prior to publication.

(1) In Abstract, the Methods are confusingly written. Were patients classified into ER+ and ER- groups, and then separately classified into genetically-altered vs. –normal
groups, or classified as “PCR vs. no pCR”?

We rewrote the sentence in the method section of abstract to avoid the confusion.

(2) In Results, 1st paragraph, the authors state that gains/amplifications are grouped and referred to as “gain” in their analyses. Does this mean that copy numbers gains were added for both tumor suppressor genes (PTEN) and oncogenes (CCND1)? If so, this would confound analysis.

We changed “genes” to “oncogenes” in the revised results (page 11).

(3) The authors note (p. 14) that there was no difference in RFS based on pCR vs. no pCR. Has this been previously reported in HER2+ breast cancer with patients treated with neoadjuvant/adjuvant trastuzumab? The authors should discuss this finding in light of prior publications.

Please refer to the response to the comment (No. 2) from the reviewer 1.

(4) In Results on p. 15, there is a section Clinical and genetic characteristics of HR+ and HR- patients who relapsed. The analyses focused on patients whose cancers recurred, and compared HR+ vs. HR-. It is unclear what the implications of these comparisons mean to clinical practice. While there may indeed be different mechanisms of resistance between HR+ and HR- tumors, the numbers in this study are not large enough to support this claim. The authors should justify the inclusion of these analyses, or consider removing from the manuscript.

Many thanks for the comments. We agreed with the reviewer’s comment pointing out that the numbers were not large enough, and removed the whole paragraph in the original results section (pages 15 and 16) and Table 6. We also deleted the relevant sentences in the abstract (page 3, lines 19 and 20) and discussion (page 19) sections.

Minor points:
(5) In Abstract, 2nd line, it is stated “a significant number of patients fail to respond…” Patients do not “fail,” the cancer fails. Please replace “patients” with “cancer”.

We changed the relevant sentence to “but a significant number of patients with the
tumor fail to respond”.

(6) In Abstract/Methods, the type (drugs) of chemotherapy should be stated.

We added “paclitaxel, cyclophosphamid, epirubicin, and fluorouracil” to the relevant sentence in the revised abstract.

(7) In Introduction, the authors mention RFS rates of 71-78%- please indicate the patient population being referenced (i.e., early-stage, metastatic).

We added “operable breast cancer” in the first paragraph of the revised background section (page 5).

(8) In Introduction, 3rd paragraph, the authors mention “discordant patients with metastatic breast cancer.” I think they are referring to “discordance: in terms of FISH vs. IHC results, but “discordance” is also being used to refer to changes in receptor status between primary and metastatic tumors. Please clarify which type of discordance is being discussed.

We added a new sentence “metastatic breast cancer patients with the discordance between IHC and HER2 copy numbers” in the third paragraph of the revised background section (page 5).

(9) In Introduction, page 6, second paragraph, after “but not in HR- patients” please note details such as “in treated with patients with neoadjuvant chemotherapy with trastuzumab, followed by adjuvant trastuzumab (plus endocrine therapy in HR+ patients).”

We added a new sentence “in patients with a HR+ but not HR- tumor, who were treated with chemotherapy with trastuzumab, followed by adjuvant trastuzumab (plus endocrine therapy for patients with a HR+ tumor)” in the last paragraph of the revised background section (page 6).

(10) I cannot find where “UPD” is defined in the manuscript.

UPD was defined as uniparental disomy in the last paragraph of page 8.
(11) There are minor grammatical errors that should be fixed prior to publication

We tried to fix the grammatical errors.