Author’s response to reviews

Title: The mitochondrial transporter SLC25A43 is frequently deleted and may influence cell proliferation in HER2-positive breast tumours

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Author’s response to reviews: see over
To editor-in-chief:

Dear Editor,

Thank you for the positive response on our manuscript "The mitochondrial transporter SLC25A43 is frequently deleted and influences cell proliferation in HER2-positive tumours". We have made every effort to address the comments and requests of the reviewers to revise the manuscript.

Following is a point-by-point response addressing the reviewer’s comments.

In response to

Reviewer – Marcella Mottolese

General comment:

We began by investigating only HER2-positive breast tumours in our search for novel deletions. After having found the common deletion of SLC25A43 in these tumours, we decided to further expand the search to include HER2-negative breast tumours as well as other malignancies. The purpose of this was simply to investigate, using LOH analysis, whether deletion of SLC25A43 could also be found in these tumours or if this deletion was exclusive to HER2-positive breast cancers. Using this technique we were able to detect deletion of SLC25A43 in these other tumours as well. However, due to the restricted amount of patient information accessible regarding these other malignancies we decided to only continue with the HER2-positive breast tumour cohort for further investigation.

In order to clarify and to highlight the main findings in the article we have added the words 'may' and 'breast' to the title: “The mitochondrial transporter SLC25A43 is frequently deleted and may influence cell proliferation in HER2-positive breast tumours”. We have also clarified the aim of this study both in the ‘Introduction’ part and in the ‘Discussion’.

Major questions and comments

1. Background

Page 3, line 9: The sentence ‘The initial response to trastuzumab…who initially respond’ is now changed.
2. Materials and Methods

a) As referred to in the text, a summary of patient characteristics and SLC25A43 expression in the HER2-positive breast cancer cohort can be found in Table 1. Also, Table 2 shows the study layout summarising all patient material included in this study. The majority of the HER2-positive breast cancer patients have been given adjuvant or metastatic treatment with trastuzumab. Unfortunately when dividing the patients into different subcategories the groups contain too few cases to make any statistical analyses regarding response to therapy, survival and time to progression in relation to SLC25A43 expression. Because of this we had previously decided not to include treatment data in Table 1. It would however be of great interest to address these questions in the future using an expanded cohort of HER2-positive breast cancer. We have now included such a sentence in the ‘Discussion’. We have also found some minor errors in table 1 regarding the column placement of the mean values. This has now been corrected.

b) As addressed earlier, the main purpose of investigating HER2-negative breast cancers, lung- and cervical cancer, was to determine whether or not deletion of SLC25A43 was an event exclusive to HER2-positive breast tumours. Since we have chosen to focus on HER2-positive breast cancer in this article we have therefore included HER2-negative tumours in the category "other malignancies".

c) Data for tumor characteristics are retrospective and were assessed at time of diagnosis at the Department for Pathology. We have now shortly described the methods used to analyze biomarkers in the manuscript (paragraph Patient material). Since these parameters have been routinely analysed in clinical setting we choose not to add the information within the Immunohistochemistry section of the manuscript.

3. Results

a) We have made every effort to address the concerns regarding the Results section as follows;

b) Page 12, line 7: Under the subheading ‘Deletion at Xq24 in HER2-positive breast cancer’. Xq24 deletion was observed in 80% of the 25 HER2-positive breast cancers that were screened using a whole-genome array. We have now reformulated line 8 to make this clear. Also, in line 10, we have changed the sentence ‘In all tumours’ to ‘In all these tumours’.


Whether similar deletion at Xq24 occurs in other cancers is not known. Deletion at Xq24 that was found the HER-2 positive breast cancers included the gene SLC25A43. Using a PCR based LOH assay we found that SLC25A43 deletion also occur in HER2-negative breast cancers, cervical cancers and lung cancers (adenocarcinoma).

c) Page 12, previous lines 15-19 has now been moved to the Discussion section and the remaining text has been changed.

d) Previous page 12, line 13 ‘The high frequency of LOH…’, we are referring to the frequency of cases with LOH in SLC25A43 as revealed by the PCR-based LOH assay.

In page 13, previous lines 3-5. The sentence, ‘There was no significant association between SLC25A43 expression and LOH … … few number of cases with LOH in the SLC25A43 gene (n=16)’ indicates the number of cases with LOH in SLC25A43 that were available for comparison with the SLC25A43 protein expression data.

In short, we started with 85 HER2-positive breast cancer cases to study LOH in the SLC25A43 gene. 33 of these cases were heterozygous for the reference SNP and therefore used for further LOH analysis. 16 of these 33 cases (48%) had LOH in SLC25A43 (Table 3).

In order to evaluate SLC25A43 protein expression by IHC, only 71 of the 85 HER2-positive breast tumours were available. Of these 71 cases, LOH data was available only for 16.

To make this clearer, we have now modified the lines 3-5 on page 13 as follows: ‘There was no significant association between SLC25A43 expression and LOH, which could in part be explained by the few number of cases with LOH in the SLC25A43 gene (n=16) that were available for comparison’.

e) Ki-67 was not analysed in clinical routine during the period of time for diagnosis regarding the HER2-positive tumours included in present study. Instead the S-phase fraction was routinely used as a proliferation marker during this period of time and with good reproducibility between laboratories (Baldetorp B et al. Cytomery 2003 Nov;56(1):1-7).

4. Discussion

The aim of this study was to identify novel copy number variations (CNVs) in HER2-positive breast cancer using whole-genome single nucleotide polymorphism arrays. The aim was of course to find novel CNVs that might cast further light on the complex mechanisms underlying HER2-positive breast cancer development and progression. The main purpose of
this study was not to discover “unexplored proteins potentially associated to trastuzumab resistance in breast cancer patients.” This has now been clarified in the end of the Background as well as in Discussion. Also, in the Discussion we have now made an effort to discuss the possible link between deletion, LOH and protein expression.

Reviewer – Liza Makowski

Major compulsory revisions

1. The HER2-negative tumors were only used in the study to assess presence of LOH in the SLC25A43 gene as comparison to our primary finding of LOH in HER2-positive tumors. Data regarding the S-phase fraction for the HER2-negative cases was not available. In the HER2-positive tumors, S-phase distribution was independent of LOH and ROH.

2. The HER2-negative tumors were not available for IHC staining and the main interest in the study was HER2-positive tumors.

3. The S-phase was measured, at the time of diagnosis in clinical routine, using a flow cytometry assay based on propidium idodide staining of the nuclei. A description is now added within the manuscript.

4. The abstract and the manuscript (see Discussion and Conclusion) are now restated to better reflect the relation between SLC25A43 protein expression and S-phase fraction in HER2-positive breast cancer.

5. Liza Makowski raises a similar question as Marcella Mottolese regarding the patient data in relation to the SLC25A43 expression. The analyses of SLC25A43 expression in association with lymph node metastasis are now better described in the manuscript, both in the Results and Discussion part. As addressed earlier, our cohort is unfortunately too small to make any statistical analyses of response to therapy, survival or time to progression. We have therefore chosen not to discuss any plausible relation between SLC25A43 expression and patient outcome in the discussion part of the manuscript.
6. In the paragraph Patient material the lung cancer cases are all defined as adenocarcinoma.

7. In response to reviewer’s comment the sentence ‘The prognostic value ….inconclusive data’ is now removed.

Kindly contact us if you have any further concerns regarding the revised manuscript.

Sincerely yours,
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