Author's response to reviews

Title: Detection of mammaglobin mRNA in peripheral blood is associated with high grade breast cancer: interim results of a prospective cohort study

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Author's response to reviews: see over
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Title: Detection of mammaglobin mRNA in peripheral blood is associated with high grade breast cancer: interim results of a prospective cohort study

Dear Dr. Norton:

Thank you for the constructive review of our manuscript submitted for publication in BMC Cancer. We have carefully considered the reviewer’s comments in revising our manuscript and I believe that we have been able to satisfactorily address all the concerns.

In this study we performed a subset analysis of the Minimally Invasive Molecular Staging (MIMS) of Breast Cancer Trial to examine the detection rate of cancer cells in peripheral blood and in bone marrow using an established 7-gene marker panel and evaluate whether there were any definable associations of any individual gene with the traditional predictors of prognosis. This study has not been previously published. This research was carried out in compliance with the Helsinki Declaration ethical principles in medical research involving human subjects. All specimens were collected through IRB approved protocols. Text in Materials and Methods has been revised accordingly (page 5, paragraph 1).

We remain very enthusiastic about this manuscript, particularly given the considerable recent interest in the role of molecular detection of circulating and disseminated tumor cells in breast cancer.
Reviewer #1 Discretionary Revisions:

Comment 1: Page 5, paragraph 2; line 2: "bone marrow aspirates were obtained on the patients left and or right": as the authors surely know a lengthy and ongoing discussion on BM sampling relates to the optimal volume and number of sites of sampling. Do they have data to support double sided sampling as a means to increase the BM positivity rate? In other words how many of the 177 patients had a double sided sampling done?

Response: Reviewer raises an important issue but unfortunately our protocol did not record if the bone marrow specimens were obtained by unilateral or bilateral sampling.

Comment 2: Page 6; line 1: "Blood and bone marrow were then shipped at room temperature ...": were there any limitations or criteria for the pre-analytical time frame?

Response: All the specimens inside US arrived in 24 hours and international shipments arrived in 48 hours. Blood and bone marrow were shipped at room temperature and processed immediately upon arrival. Text has been revised accordingly to clarify this point (page 6, line 6).

Comment 3: Page 6 paragraph 2; final line: addition of glycogen: why was this considered essential?

Response: Glycogen was used as a nucleic acid carrier to enhance RNA precipitation. Text has been revised accordingly to clarify this point (page 7, lines 4-5).

Comment 4: Page 8 paragraph 1; line 7: the authors use Ct values in stead of the Delta-Delta CT and exclude samples based on a CT > 22 for beta-2 microglobulin; Perhaps I misread but I did not find how many samples were rejected using this criterium.

Response: Approximately 10% of samples we excluded from the analysis based on this criterion. Text has been revised accordingly (page 8, paragraph 2, line 8).
Comment 5: Page 10: the observation that ERB2 signalling in PB is non-informative is much appreciated, and is indeed without any meaning unless a prior selection step.

Response: We appreciate the reviewer's enthusiasm. No changes have been made in the text.

In summary, I believe that we have been able to address the reviewer’s comments. Please do not hesitate to contact me if there are any additional concerns.

Sincerely,

Kaidi Mikhtarian, M.D.