Author's response to reviews

Title: Anthracycline-free neoadjuvant therapy induces pathological complete responses by exploiting immune proficiency in HER2+ breast cancer patients

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Author's response to reviews: see over
To the Editor of *BMC Cancer*

Dear Editor,

enclosed please find the manuscript: *Anthracycline-free neoadjuvant therapy induces pathological complete responses by exploiting immune proficiency in HER2+ breast cancer patients*, by G. Miolo et al. that we have revised according to the Reviewers’ suggestions. We are grateful to the Reviewers for their constructive remarks and we have revised the manuscript taking their comments into account.

In detail:

**Reviewer # 1**

1. In the methods section, I would like to know what exactly a cycle of weekly paclitaxel is. Patients received 3 cycles of weekly paclitaxel, is that 3 weekly treatments, or is a cycle equal to 3 or 4 weekly treatments or is there a fourth week without treatment? Need to be more precise.

This was a single arm, phase II mono-institutional study. Patients with HER2-positive locally-advanced breast cancer received neoadjuvant weekly paclitaxel (80 mg/m$^2$ on day 1, 8, 15, and 22 repeated every 4 weeks [TP]) concurrently with trastuzumab (loading dose 4 mg/kg intravenously, then 2 mg/kg weekly) for 3 cycles, followed by evaluation and, in case of clinical response, 3 more cycles were administered to obtain pCR.

2. It seems to me that the hypothesis of 20% improvement of pCR compared to chemotherapy WITHOUT trastuzumab is not necessarily relevant. The stated objective is to show efficacy of a non-anthracycline containing regimen plus trastuzumab. Therefore, it seems to me that the objective should be to show similar results of this regimen compared to anthracyclin containing regimens.

We understand the perplexity of the Reviewer, which is based on clinical evidence that has been consolidated only recently. It should be considered, however, that our clinical trial was started in 2006 when the routine use of trastuzumab in the neoadjuvant setting was not yet approved. Moreover, in the study by H.J. Burstein *et al.* published in 2003 (*J Clin Oncol*. 2003 Jan 1;21(1):46-53), the combination of weekly trastuzumab plus paclitaxel every 3 weeks yielded only 18% of
pCR. Based on these results, at that time, the use of this combination therapy avoiding anthracyclines was not highly recommended due to the relatively low rate of pCR. Therefore, we thought that a weekly schedule regimen could have improved the pCR rate, as indeed demonstrated by the present study.

3. In the discussion, the authors write that their study provides evidence that an anthracycline-free regimen can be used safely PROVIDED that trastuzumab is started early. While I agree that probably this regimen is safe, there is no evidence that the early start of trastuzumab is the essential ingredient. With the data at hand, it cannot be concluded that it is BECAUSE of early Trastuzumab that it is effective. I am not suggesting that trastuzumab should be started later but the case must be stated properly.

We fully agree with the Reviewer that, although suggestive, the results of this study do not allow to firmly conclude that an early start of trastuzumab is crucial to obtain a high rate of pCR in the neoadjuvant setting. Although several studies have investigated the timing of trastuzumab administration in relation to an anthracycline- and taxane-based neoadjuvant chemotherapy, no general consensus has been obtained so far on this debated issue. Our study suggests that weekly concurrent administration of trastuzumab and paclitaxel is a valid anthracycline-free therapeutic option for patients with locally advanced breast carcinoma. Therefore, in keeping with the Reviewer's suggestion, the sentence stating that our anthracycline-free regimen can be used safely PROVIDED that trastuzumab is started early has been rephrased in: ... provided that trastuzumab is given concurrently with a weekly taxane.

4. Regarding the cardiac toxicity I think the authors need to be careful not to overstate their case. They are looking at 46 patients. With an expected cardiac toxicity of 10-20% in patients treated with trastuzumab alone depending on the study, one would expect to see at least a few patients with decreased LVEF. While it is true that this regimen is most likely not specifically cardiotoxic I still think it needs to be stated that there were actually fewer events than might have been suspected and that this is most likely linked to the small number of patients treated.

We agree with the Reviewer's suggestion on the need to avoid overstatements with regard to the rate of cardiac toxicities observed in our cohort of patients. Several studies have indeed carefully investigated the cardiac safety of trastuzumab-containing regimens showing that most of these toxic effects are asymptomatic and detected on routine imaging, with rates of symptomatic cardiac events ranging from 1.6% to 4.1% in the major adjuvant trials.

We have therefore revised the text by including the following sentence:

“Although limited by the small number of patients enrolled and by median follow up with a wide confidence interval, we report a 8.6% rate (4 out of 46) of asymptomatic cardiotoxicity, in line with previous studies and a lower incidence of symptomatic cardiac events than expected (0.74-1.9).”

5. I am a little bit surprised not to see a larger difference in pCR between the HR negative and positive HER2 pos tumors. According to other studies, usually HR negative patients have
significantly higher pCR rates. The authors should comment about this fact (again most likely due to number, and there is a numerical difference).

The 36% difference in pCR rate observed in our study between HER2-positive and HER2-negative cases is in line with the results of the most recent studies. In fact, in the NOAH trial, a 26% difference in pCR rate between HER2-positive (43%) and HER2-negative (17%) breast cancer was detected.

6. The immune response studies are interesting and confirm the effect of the V/F genotype of the FcgRIII. The cytokine studies are equally interesting and should certainly inspire further studies to further investigate and validate the findings. I find that the authors should be careful about comparing the HER2 neg patients with the HER2 pos patients since the biology of these two tumor types is likely very different and therefore it seems difficult to compare the immune reaction to them. In addition, the treatment regimen is also completely different, since the HER2 neg tumors are not treated with paclitaxel, therefore it is not only trastuzumab that distinguishes the two groups.

We appreciate that the Reviewer considers interesting our immunological findings. Indeed, these results stimulated further analyses carried out in the same cohort of patients aimed at assessing the possible contribution of host anti-tumor immunity in mediating the clinical response induced by neoadjuvant chemotherapy. The results of this body of additional data will be included in a separated manuscript. We are well aware that the biology of HER2-positive and HER2-negative tumors is completely different, as also confirmed by our previous immunological characterization of these two groups of patients at diagnosis, before the start of treatment (Muraro et al., Breast Cancer Research 2011). Our main aim is not the comparison of immune responses between HER2-positive and HER2-negative cases, but to assess whether, within each of these two subgroup of patients, those able to mount a more effective anti-tumor immune response are more likely to obtain complete pathological responses. This is certainly more likely to occur in HER2-positive patients due to their retained immune proficiency and the use of drugs, such as trastuzumab, able to exert stronger immuno-mediated effects. Careful evaluation of our additional immunological analyses will help elucidate this intriguing issue. In line with the suggestion of the Reviewer, we have modified the Discussion section to avoid any possible misinterpretation.

Reviewer # 2

Minor Essential Revisions:

1. Introduction: line 70: [2] The reference named is from palliative situations here the context is for NC
As suggested by the Reviewer, We changed the reference with a new one more consistent with the NC context.

2. Results, Serum cytokine profiling, line 296-299 inside the parentheses: The facts are not well stated and ambiguous. Review of wording is necessary to clarify the decrease/increase of cytokines.
We agree with the Reviewer that the description of the results relative to the observed changes in cytokine levels is not immediately understandable. We have therefore carefully revised this section of the Results.

**Discretionary revisions**

*Abstract background: line 40 ...., but Is penalized by a severe cardiotoxicity WHEN COMBINED WITH ANTHRACYCLINES*

As suggested by the Reviewer, we have integrated the sentence with “when combined with anthracyclines”

**Editor’s request**

We have included the name of the clinical trial registry at the end of the abstract as requested.

We hope that our paper is now suitable for publication in *BMC Cancer*.

Yours sincerely,

Riccardo Dolcetti

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