Author's response to reviews

Title: Influence of zoledronic acid on disseminated tumor cells in bone marrow and survival: results of a prospective clinical trial

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Author's response to reviews: see over
We would like to thank the Reviewers for their constructive and insightful comments. Attached please find our point-by-point responses to the reviewers’ concerns, and a revised manuscript. All changed are highlighted in yellow.

Reviewer 1:
The paper is well written and describes a - although rather small - study which apparently was well conducted. What is missing is at least an attempt to differentiate the results by menopausal status - it is now widely accepted that this makes a huge difference for adjuvant bisphosphonate efficacy. If the claim that those effects from large clinical trials are in fact caused by Zoledronic Acid's effect on DTCs is valid, there should be at least a trend.

We have tested for an association with menopausal status but found no correlation (and no trend) with effects of ZOL stratified by menopausal status. This is probably due to small sample size.

Reviewer 2:
- Major Compulsory Revisions
  1) Material and Methods: please add statistics/power analysis made before study start.

We added this section into the manuscript.

2) Please add in the discussion the limitations of the work, eg the relative small patient number.

We extended this part of the discussion.

- Minor Essential Revisions
  1) abstract: please add study design (open-label, multicenter trial) and time frame that study has been conducted

Done.

2) Introduction, last sentence p5: change 'the effects of ZOL on DTC had not been' in 'the effects of ZOL on DTC have not been'

Changed.
3) results, patients' characteristics, 2nd paragraph: change 'clinical data' in 'patients' characteristics'

Changed.

4) Discussion, antitumorigenic effects of bisphosphonates, line 13: please change 'in this study' into 'in that study'

Changed.

5) Table 1: inclusion criteria included only patients with DTC-positive bone marrow. Then how can the DTC counts at diagnosis range between 0 and ...?

The reviewer is right. Thank you very much! We corrected the table.

6) In the discussion various possible mechanism behind the influence of Zol on DTC were summarized. Do the current data give additional information about the mechanism behind the anti-tumor effect of zoledronic acid?

Yes, we hypothesize that ZOL affects the role of bone marrow as a suitable microenvironment for DTCs.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer 3:
This study is of importance as it shows a clinical impact of bisphophonates on survival in a population of patients selected on the presence of DTC in their bone marrow. The only clinical trial based on DTC as been more recently updated and should be discussed (Diel IJ, Jaschke A, Solomayer EF et al. Adjuvant oral clodronate improves the overall survival of primary breast cancer patients with micrometastases to the bone marrow: a long-term follow-up. Ann Oncol 2008; 19: 2007-2011.)

We extended the discussion accordingly.
From a methodological point of view, why are patients with a follow-up under 8 months excluded. If these patients are lost for follow-up, then they are censored. I do not see the rationale justifying this arbitrary exclusion on this delay of 8 months. Could the authors provide the data with all included patients (96) as initially planned? They should provide a consort diagram with all the patients.

For ten patients no follow-up could be obtained. For the rest (86), the minimum follow-up was 8 months. It was not an arbitrary exclusion of patients with follow-up under 8 months – we are very sorry for the misunderstanding! We clarified it in the Methods section.

What were the hypotheses for the design of the trial to determine the number of patients to be included? The number of patients screened for DTC detection before inclusion should be given. What are the results for survival according to DTC detection at 24 months?

There was no correlation between survival and DTC persistence at 24 months. However, the sample size is very small (only five DTC positive patients).

Minor comments:

We added the reference to the AZURE trial and changed ref. 32.

Page 5, last sentence should be modified, taking into account the Aft’s paper as first sentence page 13.

We changed it accordingly.

Beginning of page 8: was it a BM biopsy or a BM aspiration? idem page 11

It was an aspiration. We changed the manuscript accordingly.

Page 13 : apoptosis of 99.9% of CT is a hypothesis and is not demonstrated. This should be mentioned.
True. We changed this sentence accordingly.

Page 17 last paragraph should be revised: no references cited, number given (23 and 18) without link.

We revised the subchapter “Clinical relevance of bisphosphonate treatment in adjuvant setting”. The references were corrected.

Table 1: median of DTC for positive cases (then range 1 to 6) or for all cases (this would be different from Solomayer’s 2012 paper).

Corrected (all included patients are DTC positive, so range should be e.g. 1-6).

Table 4: third column: yes instead of Ja?

Of course, thank you! We changed it.

Last figure is not useful and could be omitted.

Done.


Done.

First page : novartis and not norvartis

Corrected.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Additionally, please address the following editorial requests:

(1) Please include Trial Registration Number at the end of Abstract;

Done.

(2) specify the names of the Ethics Committees that approved the study;

Done.

(3) and kindly move Funding to Acknowledgment section.

Done.

(4) Authors' contributions

Done.