Peer Review File

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Reviewer A

The manuscript deals with the results in a trial with zoledronate and chemotherapy treatment of bresat carcinomas.
The authors described in details the results of this trial pointing out that the zoeldronate can increase the pathological complete response rate.
This manuscript would focus on the five years survival of patients.
There are no specific comments, However, it is not clear where and why zoledronate is effective. The previous paper from the same groups do not clarify with experiments at the bench why the treatment by adding zoledronate can be better than without.
Please try to insert explanation on this point.

Reply: We included the following on the last paragraph of the introduction
Changes in the text: "Zoledronic acid (ZA) is a bisphosphonate improved outcomes in breast cancer and it should be discussed as an adjuvant treatment.[15] The mevalonate pathway (MVP), a secondary pathway with a relevant interplay in the estrogen receptor (ER)-HER2 crosstalk, which is blocked by ZA.[16] Pre-clinical evidence suggests that ZA synergizes with anthracycline and paclitaxel.[17,18] Moreover, it can blocking the MVP, it can inhibit this pathway of resistance to anti-HER2 therapy.[19]"

Reviewer B

I commend the authors for conducting this prospective study, evaluating the role of zoledronic acid (ZA) in in improving survival outcomes in HER2 positive breast cancer. Although the study could not demonstrate a survival benefit, and is limited by a small sample size, we find the question it asks relevant. However, there are important issues that need to addressed, as follows:
Major
1. Abstract: the abstract needs to be restructured, with or without headings (as per journal guidelines) it should be introduction, methods, results and conclusion.
a. In its present state, there is no clear demarcation of methods and results. Also, endpoints need to be defined. In paragraph 2, page 1 there is mention of many secondary outcomes and a 'survival analysis' This should be changed to define RFS and OS. Focus of the results in the abstract is RFS and OS and these should be mentioned clearly.

Reply: We amended the abstract as recommended.
Changes in the text: Separating into recommended sections and clarified the main aim of this paper by highlighting the report of event-free and overall survival.

b. Page 1 , paragraph 1 line 3-5 the sentence says "more pronounced in Hormonal Receptor (HR) 24 positive, when combined with a neoadjuvant systemic treatment regimen containing Anthracycline, Taxane, 25 and Trastuzumab before surgery". Then further down in paragraph 6, line 3 there is a statement "we showed similar pCR rates in 36 both hormonal receptor (HR) positive (40%) and negative (44%).". Please reconcile these opposite statements

Reply: We amended the abstract.
Changes in the text: We removed the "more pronounced in Hormonal Receptor (HR) 24 positive, when combined with a neoadjuvant systemic treatment regimen containing Anthracycline, Taxane, and Trastuzumab before surgery" and described better the response according to hormonal receptor status.

c. It is not clear from methods that 8 cycles of chemotherapy was given. Kindly consider rewording.

Reply: We amended the abstract
Changes in the text: We clarified that 8 cycles in total were given as well as for zoledronic acid.

d. The statement "OS was equivalent according to HR status, respectively 85.7% vs. 87.5% for HR-positive and HR-negative 41 (p=0.91), which contrasted with RFS, HR-positive 81% vs. HR-negative 75% (p=0.58)" is misleading as neither OS nor RFS is statistically significant. In that sense, both are similar/ no difference seen.

Reply: We amended the abstract by emphasizing the absence of statistically significancy in the beginning of the penultimate phrase.
Changes in the text: "Although not statistically significant, OS was numerically equivalent according to HR status, respectively 85.7% vs. 87.5% for HR-positive and HR-negative (p=0.91), which contrasted with RFS, HR-positive 81% vs. HR-negative 75% (p=0.58). None of the assessed clinicopathological biomarkers significantly correlated with survival"

e. In the conclusion, paragraph 4, on page 1 the wording "Zoledronic acid plus neoadjuvant chemotherapy in HER2-positive breast cancer is associated with an encouraging survival outcomes. This combination in standard practice could be considered" needs to be more guarded as these results are thought provoking at best, and not practice changing.

Reply: We amended the conclusion.
Changes in the text: "Zoledronic acid plus neoadjuvant therapy in HER2-positive breast cancer shows provoking survival outcomes. This investigation with dual anti-HER2 blockage is warranted."

2. Many grammatical errors make reading difficult. This document will benefit from a proof read. Some examples:
a. There are multiple instances of inappropriate capitalization of words. For e.g., "Survival" as the last word in paragraph 3, page 1, "Survival" again on page 1, paragraph one of introduction etc
b. HER 2 is spelled as Her2 in introduction, page 1 paragraph 1 line 2, page 4 paragraph 2
c. The spelling for Kaplan Meier curves is wrong on page 3, figure 1 header
d. In the abstract on page 1, "amendable of" should be "amenable to surgery"

Reply: We thoroughly reviewed the paper and amended the highlighted typos.

e. All abbreviations need to occur in their full form the first time they occur in the abstract AND the manuscript. The abstract is a stand-alone document and abbreviations expanded there do not count for the manuscript. For e.g., HER2

Reply: We thoroughly reviewed the paper and amended as recommended.

f. It is difficult to understand the frequency with which zoledronic acid was given. It appears that it was given monthly, but ' a week after each chemotherapy session' could also mean every 3 weeks – which is a shorter frequency than recommended for this drug?

Reply: We amended the frequency in the abstract and in the text to clarify that 8 cycles of zoledronic acid were given alongside each cycle of chemotherapy. We also would like to highlight that Zoledronic acid can be given every 3 weeks as per label accessed at the time the study was designed and conducted. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021223s028lbl.pdf

3. Also, in figure 1b on page 7, it appears that the blue line('no pCR') has a superior RFS than the green line(pCR). This is not explained by the results documented. This needs to be rechecked.

Reply: We amended the second paragraph of the discussion by adding information in the end.
Changes in the text: "It is important to highlight that we believe the numerically higher but not statistically significant RFS for HR-negative patients non achieving pCR than achieving pCR are likely to be attributed to the small number of subjects. (Fig 1D)"

4. I see that relevant studies have been included for comparison in table 1. I do feel, however that the readership experience can be improved by adding some lines about these studies. For example, in the TRYPHAENA study the pCR with each arm was actually around 65%, which, at the risk of cross trial comparisons is better than the current study. It can also be discussed that although many studies like the current one demonstrate pCR improvement with augmented neoadjuvant regimens, none have been able to show a survival benefit. Moreover, in some of these studies in the table, the anthracycline part of chemotherapy was delivered after the surgery(such as the Neosphere study). These points need to be addressed in the discussion or limitations section for a fair comparison.

Reply: We amended the text by adding this information as a second phrase of the penultimous paragraph of the discussion.
Changes in the text: "Higher pCR rates were achieved with regimens including dual anti-HER2 blockage and anthracycline before surgery, such as in the TRYPHAENA trial, but similar when anthracycline was given after surgery, such as in the NEOSPHERE trial, as summarized on Table 1."

5. The authors have commented on similar outcomes with other chemotherapy regimens, but have they compared the baseline risk of the patients included in these trials? Do we know if these patients had a similar risk profile(stage, age, grade, receptor status etc) to other patients?

Reply: We added to the second phrase of the discussion
Changes in the text: "in our population, > 50% with both clinically positive lymph-node and stage III".

Minor
1. Page 3 paragraph 2 consider rewording "and the high-level results were presented at the ESMO-Breast conference in 2023" to a more conservatively stated sentence

Reply: We re-worded to a better adjective
Changes in the text: "the preliminary".

2. In table 1, on page 7 the headers are labeled as mOS and OS in different columns. Should be any one of them but not both. Similarly, throughout the results in the article, the endpoint mentioned is RFS but the labelled header in the table is DFS. If these are being used interchangeably, this needs to be addressed in the text. The different fonts of table 1 also need to be resolved to a single font.

Reply: We appreciate the comment, however few studies report median OS and other studies report OS rate at the specific timeline.
Changes in the text: For the OS, we amended the footnotes of Table 1 to translate it more accurately and adjusted the font as requested. Moreover, we amended the headings from "DFS" to "RFS"

3. Figure: On page in the figure 1 header ". RFS is controlled by pCR in HR+ (C) 327 and HR-" this does not correlate with the results obtained. Need to rephrase.

Reply: We amended the figure header
Changes in the text: The word "is" was removed.

4. Typically, the limitations section should be labelled. I would also include small sample size and the fact that it is a phase 2 single arm study; therefore, as per limitation #6, cannot do a cross-trial comparison

Reply: We amended the limitation paragraph, which now preceded with a heading called "limitations".

Changes in the text: The following was included as a second phrase “Our small single arm study limits the comparison with studies.”, including “ZA +” before “the dual-blockage” and added the following to the ante-penultimate phrase as following “and equally to our design, led to limitations in assess survival benefit”.