Peer Review File

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Reviewer A

Axillary Surgery (MUTAS) trial, ClinicalTrials.gov Identifier: NCT04039893

I congratulate the authors on taking up the project to assess the accuracy of sentinel node mapping in patients with biopsy-proven metastatic axillary lymph nodes and upfront surgery. The study is well-planned and well documented.

I do have the following suggestions for the authors:

1) In the Methods section in the Abstract it maybe be worthwhile to mention the duration of study and that the preliminary phase was conducted at a single center.

Reply 1: We thank the reviewer for this observation and are in agreement with it.

Changes in the text: We have added the information required in the abstract. We have changed line 50 and 51 page 3 as follows:

Between September 2019 and March 2022, we recruited patients with proven metastatic axillary nodes and upfront surgery from a single center, Hospital del Mar in Barcelona, Spain.

2) In the Methods section, under Recruitment it may be worthwhile to clearly mention any exclusion criteria.

Reply 2: We agree with the reviewer that the exclusion criteria should be stated.

Changes in the text: We have included the exclusion criteria in line 130, page 6, between "were invited to participate in the trial" and "Before inclusion" as follows:

Exclusion criteria were a previous history of breast cancer in the same or the contralateral breast, patients aged <18 years, inability to understand the trial, and non-proven metastatic axillary lymph nodes on either cytology or biopsy.

3) It may be useful to describe if Blue Dye was used in any patient if gamma probe did not catch a good signal.

Reply 3: The authors thank the reviewer for reminding us about this. In our Breast Unit we never use Blue Dye in patients treated with upfront surgery. Consequently, we did not include this as an option in the trial. We agree with the reviewer that it would be informative for the readers to know if Blue Dye was used at any moment.

Changes in the text: We have changed lines 135 and 136 on page 6. Following the sentence "Trial participants underwent sentinel node mapping according to the standard protocol in our Breast Disease Unit," we have added:

where we perform the sentinel node exclusively with radiotracer in patients treated with upfront surgery.

4) A little clarification on the sample size calculation would be helpful. Line 201 and 202 state

that "we expected a false-negative rate of 20%. To prove this difference in a unilateral test, with a 95% confidence level and an 85% statistical power, 59 patients were deemed necessary." At 20%, the power should be 80% as against the 85% statical power mentioned. Also, since Mann Whitney U test is a non-parametric test it maybe worthwhile to mention that the continuous parameters are non-normal in distribution and have been assessed for.

Reply 4: We apologize for the error, writing 85% was a transcription mistake. Also, we agree that it must be stated that continuous parameters were non-normal in distribution.

Changes in the text: We have changed line 202 page 9, so now the sentence is "To prove this difference in a unilateral test, with a 95% confidence level and 80% statistical power, 59 patients were deemed necessary."

Also, in line 221 page 10, after "Continuous data were compared using the Mann–Whitney U test" we have added:

"as continuous parameters were non-normally distributed."

5) It maybe worthwhile to rephrase and club features of the tumor together and lymph node together in lines 214-218 just to ensure ease of understanding.

Reply 5: The authors agree with the reviewer; features are not clubbed, which makes the text difficult to understand for the reader.

Changes in the text: We have rephrased the whole sentence in lines 214-218 as follows: "Based on the pathological report, we assessed as predictive variables of the tumor, the percentages of ER expression, PR expression, the Ki67 percentage score, lobular carcinoma as a pathological type, histological grade III, the presence of lymphovascular invasion, the size of the largest focus of invasion and total tumor size; as predictive variables of the lymph nodes, we assessed the size of the abnormal node identified, the number of sentinel nodes excised, the number of remaining axillary nodes excised, and extranodal extension."

6) In the section on Predictive factors of involvement of the remaining axillary nodes, false-negatives and false- positives (line 204), it doesn't mention any previous history of breast or axillary surgery/radiotherapy or if tumours were multifocal - these may influence the localisation of sentinel nodes.

Reply 6: We agree with the reviewer that previous treatments in the breast or the axilla or multifocal tumors could affect sentinel nodes identification. None of the 25 patients analyzed had previous treatments in the axilla or the breast and only one patient had multifocal disease. This is the reason we didn't include these features in the assessment of predictor factors.

Changes in the text: We have added this information to the manuscript. After line 323 in page 14, following the sentence "Although we report some differences in the percentages in this bivariate analysis, most of them were non-significant." We have added the following text:

In addition, because of the low number of cases analyzed, we did not evaluate other important features as predictors of involvement of the remaining axillary tissue, such as tumor multifocality, because they were sparsely represented in this cohort."

7) It maybe worthwhile to re-phrase lines 236-240 as the current wording is not very easy to understand.

Reply 7: We agree with the reviewer. This sentence is not giving the information in a clear way

as we tried to include too many details.
Changes in the text: We have changed the sentences between the lines 236 and line 240 in page 10. Now the text is as follows:

Of the 25 patients, the biopsy-proven metastatic lymph node was not a sentinel node in 4 (16%): of these 4 patients, no other sentinel nodes were identified in two, 2 sentinel nodes were identified in one and 3 sentinel nodes were identified in the remaining patient. Of the two patients with no identified sentinel nodes, only one showed infiltration of the remaining axillary nodes.

8) It maybe helpful to follow same numbering format – Table 1 and Table II (line 260 and 265)
Reply 8: We apologize for the error. This was a transcription error, thank you very much for your correction.
Changes in the text: We have changed the numbering in all the tables so that now they are all Arabic and none is Roman.

9) SN needs to be defined in the legend of Table 2 legend
Reply 9: We apologize for the omission, which we did realize was missing in our final revision before sending the manuscript. Thank you very much for pointing this out.
Changes in the text: In line 498 page 24 we have added this information, so now it reads:
"SN, sentinel node; LN, lymph node; FN, false negative; FP, false positive; SD, standard deviation; US, ultrasound; ER, estrogen receptor; PR, progesterone receptor; DCIS, ductal carcinoma in situ."

Reviewer B

This is a pilot study dealing with the reliability of sentinel node biopsy in clinically node positive patients undergoing upfront surgery. The topic is very important, with high clinical relevance, and I congratulate the authors for their brave work. The paper is well written, I have a few comments:

1. In the introduction line 85- instead of- 'could undergo breast-conserving surgery' I suggest- 'underwent breast-conserving surgery and could receive adjuvant radiation therapy'
Reply 1: The authors thank the reviewer for the observation, and the change proposed makes the description of the Z0011 trial much more accurate.
Changes in the text: As suggested, in line 85 and 86 page 4, we have changed the sentence "who could undergo breast-conserving surgery" for the following sentence:
"who underwent breast-conserving surgery and could receive adjuvant radiation therapy",

2. Line 88-89- 'the trial did not include the results of axillary ultrasound'- I suggest adding the word 'preoperatively' before axillary US.
Reply 2: The authors thank the reviewer for this observation. Adding this information makes the description much more precise.
Changes in the text: We have changed the sentence in line 85 page 4, so now it reads:

"the results of preoperative axillary ultrasound".

3. Technically- have the authors considered marking the biopsy-proven LN preoperatively with a wire or tattoo?

Reply 3: The authors thank the reviewer for the suggestion. We presently do use a wire when we are unable to detect the ultrasound visible marker in the preoperative evaluation of the patient. Sometimes we have encountered difficulties during the intervention, as a marker that had been detected in the preoperative evaluation could not be detected thereafter intraoperatively, probably because the ultrasound visible marker slipped out during the first steps of the dissection. At that moment, we found ourselves with no alternative to detect the marked lymph node. This is what happened with some patients in the trial that finally had to be excluded.

Regarding the tattoo, we have never used it in our Breast Unit to identify infiltrated lymph nodes, but we have read that other authors have good results with it and we shall start using it in the near future.

Changes in the text: We have made no changes in the text as no changes are suggested.

4. In the Results- we excluded 7 (in 4, the intervention was postponed due to a positive Covid screening test when the sentinel nodes had already been identified and they were excluded to avoid them undergoing a second procedure'- please clarify- what do you mean by 'sentinel node already been identified'?

Reply 4: We agree with the reviewer that in this sentence it is not clear what we mean by stating that the sentinel node had already been identified. What we really meant is that the preoperative lymphoscintigraphy had already been performed. We thank the reviewer for this correction.

Changes in the text: In line 229-231 page 10 we have changed the sentence "in 4, the intervention was postponed due to a positive Covid screening test when the sentinel nodes had already been identified" and now it reads as follows:

"in 4, the intervention was postponed due to a positive Covid screening test when the preoperative lymphoscintigraphy had already been performed"

5. The classical 'Table 1' is missing- who are the patients? What are their clinical, demographic, and histologic characteristics? All we know is age and the fact that all were ER positive HER2 negative.

Reply 5: The authors thank the reviewer for the observation. We agree that adding a descriptive table would enrich the manuscript.

Changes in the text: We have added a descriptive table, identified as Table 1. We have mentioned the existence of this table in line 247 page 11, adding the following sentence:

"The clinical, imaging and pathology features of the patients are summarized in Table 1."

6. It seems that this cohort does not represent the z11 cohort, not only in regard to the relatively high number of involved nodes, but also the large tumor size (mean 43 mm, almost T3, which weren't included in the z11), and the fact that all tumors were luminal (obviously, otherwise they would go for NACT)- the authors should mention these limitations of the study.

Reply 6: We agree with the reviewer that the size of the tumors is a very significant factor

regarding the number of infiltrated axillary lymph nodes. In the Z0011 trial, the median and range of the tumor size was 1.7 (0.4-7.0) cm for the Axillary Lymph Nodes Dissection Group and 1.6 (0.0-5.0) cm in the Sentinel Node Group. As we reported the mean size of the tumors instead of the median, as in the Z0011 trial, the figures are hardly comparable. We see now that we should report our results as a median, as the standard deviation is bigger than the mean itself. Nevertheless, the mean in our study is 22.55, range 3.1-130 mm, higher than in the Z0011 trial. We have included this weakness in the Discussion section.

Changes in the text: We have changed line 234 and 235 page 10, so that instead of "the mean tumor size in the pathology report was 42.92 (SD 55.49) mm" now it reads as follows:

"the median tumor size in the pathology report was 22.55, range 3.1-130 mm".

Also, in the Discussion section, line 297 page 13, after the sentence "which is substantially higher than the 21% reported in Z0011" we have added the following sentence:

"This difference could be attributed to the fact that the median tumor size is larger in our study."

7. In the results lines 236-252- perhaps a graphic way to show these results will be easier to follow.

Reply 7: We agree with the reviewer that the way we have reported these results in lines 236-252 is quite confusing. As another reviewer made the same observation, we have rephrased this sentence. We hope this will be satisfactory.

Changes in the text: We have changed the sentences between lines 236 and line 240 on page 10. After the rephrasing the text is as follows:

"Of the 25 patients, the biopsy-proven metastatic lymph node was not a sentinel node in 4 (16%): of these 4 patients, no other sentinel nodes were identified in two, 2 sentinel nodes were identified in one and 3 sentinel nodes were identified in the remaining patient. Of the two patients with no identified sentinel nodes, only one showed infiltration of the remaining axillary nodes."

8. The term 'False positive' here is somewhat a misnomer as the original purpose of performing SLNB was to assess the status of the axilla- positive or negative, not to predict the remaining nodes status. Also, technically we know that sometimes there is an adjacent node that's being removed together with the sentinel node and may be counted as a sentinel node, therefore this separation is not completely accurate. We know from the z11 that in the group who underwent SLN only- additional positive nodes were probably left behind, and this fact did not change the study endpoint- OS and regional recurrence, so the question is not how many non-sentinel nodes were positive, but whether SLNB is reliable in patients with clinically involved node, (in other words whether the z11 is applicable in these patients) therefore the FNR is what we are interested in.

Reply 8: The authors thank the reviewer for this wise observation. As stated, a false positive in the classical scenario would have not much meaning, as a single positive sentinel lymph node means a positive axilla.

When planning the trial, one of the points for us was that the Z0011 recommended axillary lymph node dissection if more than 2 sentinel nodes were positive. Consequently, the Z011 trial put value on the fact that a precise number of positive sentinel nodes was able to predict the status of the rest of the axillary lymph nodes and consequently the axillary tumor load.

Therefore, the question for us was mainly which was the percentage of false negatives, but also which was the percentage of false positives in this new Z0011 scenario with this new perspective.
In the Material and Methods section, we defined what the false positive sentinel node meant in our trial when adopting this somewhat new indication of the sentinel node technique: line 192-193 page 8, "false-positives were defined as cases with at least one metastatic sentinel node but no involvement of any of the remaining axillary nodes." Again, you are right that this is a different concept not appliable to the routine clinical use of the sentinel node in patients with upfront surgery.
Changes in the text: We have made no changes as no changes are requested.

9. The authors did not use Blue dye, perhaps from ethical reasons, however this might affected the reliability of the SLNB procedure, as we know from other scenarios that dual mapping can lower FNR. (Also, 2 patients had no mapping at all.)
Reply 9: This is a very appropriate observation. In our Breast Unit we never use Blue Dye in patients treated with upfront surgery, and this is the reason why we did not include it as an option in this pilot phase of the trial. For the next steps of the MUTAS trial, the option of using blue or some other dye (i.e., fluoresceine) shall be considered.
Changes in the text: We have made no changes as no changes are requested.

10. Figure 1 and 2 are visually blurred- impossible to read.
Reply 10: We are really sorry for that. We will send them again ensuring that they are of sufficient quality.
Changes in the text: We have made no changes as no changes are requested.

Reviewer C
I thank you for the opportunity to review this article.

In general: the topic researched in the article is important, and the study is well planned and executed, but the number of patients included in the study is far from what should be considered appropriate and the authors fail to prove the aim of the study, which was to establish the accuracy of sentinel node mapping in patients with axillary involvement.

I have several concerns, which the most important are the following:

1. the study included only patients with biopsy-confirmed axillary metastasis. The study does not include patients with suspicious axillary lymph nodes, which prove not to be metastatic. There should be some kind of number of these patients, since it is a critical question whether these lymph nodes should be biopsied at all.
Reply 1: The subject mentioned by the reviewer is important and we understand the concerns. However, it would have been unethical from our point of view to include patients with no confirmation of the metastasis by cytology or biopsy because an axillary lymph node dissection was planned for all the patients included in the trial.

For now, in our Breast Unit, all ultrasound suspicious axillary lymph nodes are studied by cytology or biopsy. If they turn out to be positive and upfront surgery is decided, we counsel the patients to go through an axillary lymph node dissection; these are the patients included in the trial, in whom a sentinel node was additionally performed, but the final intervention was routine axillary dissection.
If the cytology or the biopsy turns out to be negative, we counsel the patient to perform a sentinel node and we excise the ultrasound suspicious node in case it is not a sentinel node; in this last scenario, more than 50% of the patients show no infiltration of any of the nodes.
If we had included these patients in the trial, several of them would have received an axillary dissection with a non-infiltrated axilla. Consequently, we decided to include only patients with cytology or biopsy-proven infiltrated axilla.
Changes in the text: We have made no changes as no changes are requested.

2. the comparison in the study is made to Z0011 trial, which is probably appropriate, but the Z0011 did not include palpable axillary metastasis, and most of the patients in the present study have palpable metastasis. Thus, these patients cannot be directly compared to each other. It is highly probable, that patients with palpable metastasis have a higher burden of metastasis in axilla.
Reply 2: We absolutely agree with this observation. In our study, 14 of 25 patients had palpable lymph nodes and consequently they did not meet the Z0011 trial criteria. Despite this, we decided to include these patients in the trial to study the performance of the sentinel node in this particular group and compare it to the performance of the sentinel node in patients with non-palpable lymph nodes.
As known, palpation accuracy is low and explorer-dependent, so we believed that having some data on patients with palpable nodes could provide useful information.
Changes in the text: We have made no changes as no changes are requested.

3. In the present study, the biopsy-confirmed axillary metastasis was a sentinel node in 21 of 25 patients. Therefore, it is highly questionable whether any of these patients may be considered to have a false-negative SLNB.
Reply 3: We thank the reviewer for this wise observation. It is correct that for routine use of the sentinel node, the patients included in our trial could never be considered false-negative. In the clinical scenario, the sentinel node is used when no confirmation of axillary infiltration exists, precisely to confirm axillary status by pathology and with the highest certainty available. What we proposed in our trial was a different research scenario. The axillary status of the patients included in our trial had already been confirmed as positive by a pathology exam. Consequently, the classical indication for a sentinel known, that is, knowing if the axilla was positive or negative, would have made no sense for these patients.
What we hypothesized was that the sentinel node could be helpful in predicting the axillary tumor load (infiltration of the rest of the axillary nodes other than the sentinel nodes). This is the reason why we defined the false-negatives and the false-positives in a new way suitable to our hypothesis in the Material and Methods section.
Changes in the text: We have made no changes as no changes are requested.

4. The absolute number of patients is so small, that it is deceptive to present percentages, especially without confidence intervals. For example, 4/9 is indeed 44.44%, but a single patient has a weight of 11.11% and random effect has an excessive impact on the percentages.
Reply 4: The reviewer´s observation is completely right. As we have acknowledged in the Discussion section, the low number of recruited patients is a major flaw of our study.
However, the trial has been going on for three years (two and a half during the Covid pandemic) and we thought it was already time to explain our experience in this field, as no trial like this one has ever been done.
No definitive conclusions can be drawn with such low figures, as the reviewer correctly points out, but the results suggest that the sentinel node could be a useless and inaccurate technique in the scenario we have been working on, that is, a confirmed infiltrated axilla. We believed that this information could be useful for other surgeons.
Changes in the text: We have made no changes as no changes are requested.

5. Statistical comparison to Z0011 trial results would prove the patient number in the present study to be insufficient. For example, the authors claim that "non-palpable metastatic lymph nodes…. this percentage was 36.36%, which is substantially higher than the 21% reported in Z0011." – lines 295-297. This conclusion cannot be made without statistical analysis, which should yield a p-value of 0.20 or so as the number of patients is so limited.
Reply 5: We agree with the reviewer that no direct statistical comparison is to be made between the Z0011 trial and our trial. The Z0011 is a powerful trial and ours is just a pilot study.
Changes in the text: We have changed line 293 page 12. Instead of "Of note, all of the subgroups studied in this trial had a considerably higher percentage of…" now it reads as follows:
"We acknowledge that a direct statistical comparison between our trial and the Z0011 trial would be inappropriate due to the low number of patients that we have recruited. Nevertheless, our figures show that all the subgroups studied…"

6. The authors aim to define many statistical figures from very limited number of patients, which inevitable leads to situation, where a single patient is included in multiple subgroups.
Reply 6: The reviewer is right again regarding the fact that a single patient is included in multiple groups. Nevertheless, as a pilot study attempting to identify which different scenarios should be considered in a future multicenter trial, we analyzed data from different points of view, searching for the lowest false negative rate. This is the reason why different subgroups were studied and patients could belong to one or other, depending on the features considered, as one feature did not exclude the presence of another.
Changes in the text: We have made no changes as no changes are requested.

7. The authors have chosen to include the two patients with undetectable lymph nodes in the statistics. These patients should be excluded from the analysis.
Reply 7: We understand the reviewer´s concerns regarding the two patients with undetectable lymph nodes. In fact, this point led to a long discussion between the members of the research team. Finally, as the hypothesis of our research was that finding positive sentinel nodes was predictive of infiltration of the remaining axillary tissue, and in these two cases no positive

sentinel nodes were found, we decided to consider them as part of the negative sentinel nodes group. We erroneously expressed this decision as "arbitrary" in the manuscript.
Changes in the text: We have changed lines 240-244 page 10. Instead of "Given that in these 4 patients, in whom the biopsy-proven metastatic lymph node was not a hot node and did not have a homogeneous pattern of invasion, we arbitrarily decided to include the 2 patients without additional sentinel nodes in the group of patients with negative sentinel nodes: one was a false-negative and the other was a true-negative" now it reads as follows:
"Given that the hypothesis of our study was that the presence of infiltrated sentinel nodes (other than the cytology- or biopsy-proven infiltrated lymph node) predicted the infiltration of the remaining axillary nodes, and no infiltrated sentinel nodes were identified in these two patients, we decided to include them in the group of patients with negative sentinel nodes: one was a false-negative and the other was a true-negative.

As a minor comments:

- The mean of the number of axillary metastases is not informative, as the count does not follow any specific distribution and there usually are patients with high number of metastasis (skewing). The form the authors present this information in line 246 is more appropriate, but it would be clearer to provide this information in a Table form, not in a text which is loaded with various data. This makes the text difficult to be followed.
Reply: We thank the reviewer for this observation and agree with it. We have included a descriptive table that we have identified as Table 1. We hope the information will now be easier to follow.
Changes in the text: We have changed lines 245-246 page 11. Instead of "The mean number of metastatic lymph nodes was 3.08 245 (SD 2.01): 7 patients showed 1metastatic node, 4 patients showed 2, 6 patients showed 3, and 8 patients showed 4 or more." now it reads "The clinical, imaging and pathology features of the patients are summarized in Table 1"
We have added a new table identified as Table 1.

- line 235 – the standard deviation is greater than the mean tumour size, which should imply that the information should be provided in some other form, as median and interquartile range.
Reply: The reviewer is right again. In this case it is much more informative to express the information as a median.
Changes in the text: We have changed lines 234-235 page 10. Instead of "the mean tumor size in the pathology report was 42.92 (SD 55.49) mm" now it reads as follows:
"the median tumor size in the pathology report was 22.55, range 3.1-130 mm."

Conclusion: the topic is important, and the article is mostly well written. However, the authors fail to show any contradiction to the Z0011 trial or provide any other new information, which is due to the very small number of patients. With such a small number of patients, it is unclear how the results could be presented in a form that would add something to the current literature.
Reply: We fully understand the reviewer´s concerns regarding our manuscript. With these results we do not pretend to establish new rules regarding the management of breast cancer patients with axillary infiltrated lymph nodes.

However, we believe that the results worth disseminating because they suggest that caution is necessary before decisions are taken without sufficient evidence. We and others consider premature the recommendation of the NCCN guidelines regarding the appropriateness of performing the sentinel node technique when there are one or two suspicious nodes on the axillary ultrasound. Perhaps they are not wrong, but they are premature before new well-designed trials draw definitive conclusions. The publication of our manuscript could help in fueling this new necessary research.

Reviewer D

This article describes the FNR of TAD in cases with one or two axillary lymph node metastases. We believe this paper is useful because it cautions against hasty omission of axillary lymph node dissection.

1. Although ultrasound is used to determine axillary lymph node metastasis, please describe the criteria used to determine axillary lymph node metastasis.

Reply 1: We thank the reviewer for this comment. The reviewer correctly points out that we have not explained the criteria used by our radiologists to study the axillary lymph nodes. We have now added this information in the Material and Methods section.

Changes in the text: We have changed lines 124-127 page 6

We have changed the sentence " All patients with a first diagnosis of breast cancer underwent routine axillary ultrasound in the Breast Diseases Unit of the hospital as part of disease staging. For this pilot phase, when axillary involvement was suspected on ultrasound, the number of nodes presumed metastatic were quantified."

Now it reads as follows:

"All patients with a first diagnosis of breast cancer underwent routine axillary ultrasound in the Breast Diseases Unit of the hospital as part of disease staging. Axillary lymph nodes were considered suspicious and biopsied if they showed features of type 4 (generalized lobulated hypoechoic cortex), type 5 (focal hypoechoic cortical lobulation) or type 6 (hypoechoic node with absent hilum) categories of the Bedi classification. For this pilot phase, when axillary involvement was suspected on ultrasound, the number of nodes presumed metastatic was quantified."

2. "we believe the results deserve to be reported as they may guide the design of further trials on axillary surgery that may be performed in the near future ." but please describe any specific plans.

Reply 2: We thank the reviewer for this comment. We have added in the Discussion section a brief description of the next steps we are planning for the MUTAS trial.

Changes in the text: After line 339 page 14 we have added the following text:

"Regarding the MUTAS trial, and after the results of this pilot phase, we are planning a multicenter study with techniques other than the sentinel node to identify infiltrated axillary lymph nodes."

3. In Table 3, in > 1 suspicious axillary node on US, 8/5 (61.5) should be changed to 5/8 (61.5).

Reply 3: We thank the reviewer very much for pointing out this error.

Changes in the text: In the former Table 3, which is now Table 4 as we have included a new table, we have changed "8/5" and now it reads "5/13", that are the correct figures.

Reviewer E

Accuracy of sentinel node mapping in patients with biopsy-proven metastatic axillary lymph nodes and upfront surgery is a study evaluating the outcomes of the sentinel node biopsy (slnbx) procedure in the setting of metastatic disease. This study addresses the NCCN guidelines that describe slnbx in sonographically suspicious nodes, in light of the Z11 data:

This is a needed investigation and provides some preliminary data from the MUTAS trial. I do have the following comments and questions about this manuscript:

1. The terminology of "ultrasound diagnosis of axillary involvement" first stated in the abstract (line 57) is confusing. Ultrasound can suggest lymph node involvement but does not diagnose. Only tissue/ biopsy can confirm. Please make this distinction clear in the manuscript. Would specify the study inclusion criteria in the methods section of the abstract that "biopsy" proven metastatic axillary nodes and upfront surgery.

Reply 1: We thank the reviewer for this correction and are absolutely in agreement that the diagnosis is made by the pathology exam and ultrasound draws only suspicion. Also, we agree that including the word "biopsy" in the Methods section of the Abstract would make the content clearer.

Changes in the text: We have changed lines 56-57 page 3. Now it reads as follows:

"in those with non-palpable nodes and an ultrasound that led to the diagnosis of axillary involvement"

We have also added the word "biopsy" in line 50 page 3, now it reads:

"we recruited patients with biopsy-proven metastatic axillary nodes and upfront surgery"

2. For the methods section those patients recommended to have upfront surgery, what were the clinical reasons for this in place of neoadjuvant therapy? What proportion of patients identified with nodal involvement had surgery first?

Reply 2: We thank the reviewer for this question. This is a very important point. In the protocols of our Breast Unit, receptor-positive Her2-negative breast cancers receive neoadjuvant treatment if the tumor is bigger than 3 cm or the axilla is N2, as tumors smaller than 3 cm and a limited number of infiltrated lymph nodes may benefit of performing Oncotype to decide the use of chemotherapy. In the last year, about 50% of the patients received upfront surgery. Even though this is interesting information, as it was not in the scope of our research, we decided not to include it and to focus on the accuracy of the sentinel node in this scenario.

Changes in the text: No changes have been made as no changes are requested.

3. Any consideration of using dual tracer to reduce the false negative rate of the sentinel lymph node procedure given that Technetium along is used? Any experience with targeted slnbx also with wire location or other techniques? ? Any nuances with nodal assessment during COVID pandemic. Data shows the virus and even vaccine produces adenopathy which impacted clinical

exam and radiographic appearance of nodes.
Reply 3: We thank the reviewer for pointing out these important subjects. For the trial, we decided to perform the sentinel node as we usually do it in patients with no proven axillary metastases, and we routinely use only radiotracer. We have sometimes used blue dye, but the experience was not so good. We have also sometimes used a wire to locate the infiltrated node, but the team feels more comfortable with ultrasound visible markers.
Regarding changes to the lymph nodes because of Covid or Covid vaccines, our radiologists had to deal with it as did radiologists all over the world, but if any of the enlarged Covid lymph nodes were biopsied, no infiltration was proved, so the patient could not be included in the trial. We do not have any figures on how many times this could have happened.
Changes in the text: No changes have been made as no changes are requested.

4. In results: line 236 add "her-2" along with c-erB2 as that is most commonly used
Reply 4: We thank the reviewer for this comment. We agree that Her2 is most commonly used
Changes in the text: We have added "Her2" in line 236 page 10, so now it reads "All participants were hormone-receptor-positive and c-erbB2 Her2 negative."

5. In the discussion, line 302 caution about the NCCN guidelines is reasonable. However, this study is limited by the small sample size
Reply 5: The reviewer is right to point out that our study is limited by the small sample size, so only suggestions but no conclusions can be drawn after our results. In line 302 page 13, "Consequently" refers to the metanalysis by Ahmed and the studies mentioned in the previous paragraph. We have changed the sentence to make it clearer.
Changes in the text: We have changed line 302 page 13 so that instead of
"Consequently, we believe the recommendation of the National Comprehensive Cancer Network(NCCN) guidelines…" now it reads:
"Considering all the published studies previously mentioned, we believe the recommendation of the National Comprehensive Cancer Network (NCCN) guidelines…"

Reviewer F
The submitted manuscript reports data for a small single-arm trial regarding patients with 1 or 2 suspect nodes before primary surgery for EBC and the value of SLNB in this situation. The authors state (correctly) that the NCCN recommendation regarding the use of SLNB in patients with 1 or 2 suspect nodes is disputable. In their trial tehy demostrate that FN rates are inacceptably high in this population. These data are very interesting and the manuscript is definitely worth publication. I have two comments:

1. The authors are always stating that the submitted data are from the pilot phase of the MUTAS trial. What is planned now after the pilot phase has been finished??
Reply 1: We thank the reviewer for their interest in the MUTAS trial. We are now planning a multicenter study with different methods to identify infiltrated axillary lymph nodes.
Changes in the text: After line 339 page 14 we have added the following text:
"Regarding the MUTAS trial, and after the results of this pilot phase, we are planning a

multicenter study with techniques other than the sentinel node to identify the infiltrated axillary lymph nodes."

2. The included patients had involved lymph nodes but were assigned to primary surgery. I assume the tumor biology was favorable but I cannot derive that from the tables. The HER2 status is completely missing and it is impossible to calculate the tables back to proper "patient characteristics". Please include a table with patient characteristics so we can see wat population you are talking about.

Reply 2: We thank the reviewer for this acute observation. We have included a table, identified as Table 1, describing the clinical, imaging and pathology features in these patients.

Changes in the text: We have changed lines 245-246 page 11. Instead of "The mean number of metastatic lymph nodes was 3.08 245 (SD 2.01): 7 patients showed 1metastatic node, 4 patients showed 2, 6 patients showed 3, and 8 patients showed 4 or more." now it reads:

"The clinical, imaging and pathology features of the patients are summarized in Table 1"

We have added a new table identified as Table 1.