Dear Editorial Team,

Thank you for sending the latest comments related to the manuscript MS: 1888413221000263 by Leissner et al. We were pleased to learn that the manuscript is now acceptable for publication after some last modifications. We found the comments by the referees helpful to clarify some specific points in the manuscript. Below, we summarize the specific changes and comments on all of the issues raised.

Reviewer: Dan Tong

Minor essential Revisions

1) We agree with the reviewer that the microarray results are no longer relevant in the new manuscript. So, as requested, we removed the results and the text related to the microarray data. Modifications have been made accordingly in the "RT-PCR analysis of uPA and PAI-1 gene expression" section (page 10). Figures 1A and 1B displaying correlations found between microarray and RT-PCR have been replaced by PAI-1 mRNA expression levels between relapsed and non-relapsed breast cancers (Figure 1A) and between survivors and patients that died of breast cancer (Figure 1B).

After removing the results from microarray in the manuscript, we estimate that the deposition of our microarray data in a public repository is no longer necessary.
2) As requested by the reviewer, we added the uPA/PPIB and PAI-1/PPIB mRNA ratio corresponding to the expression medians allowing to generate “high” and “low” level groups. The following sentences have been modified on page 11: "We investigated the prognostic value of uPA and PAI-1 mRNA levels by considering the median of expression as a cut-off generating a "high" and a "low" level group. The medians used corresponded to a PAI-1/PPIB and a uPA/PPIB expression ratio of 0.25 and 2.12 respectively."

3) As requested by the referee, we added the hazard ratio for the univariate analysis in Tables 3 and 4.

4) In the same way, we deleted redundant information in the text regarding the hazard ratio of the multivariate analysis (pages 11 and 12).

Discretionary Revisions

1) As suggested by the referee, the term "clinico" was replaced by "clinical" in page 2 and 11

2) We deleted "low" and "longer" words in the sentence page 2 as requested.

3) Modifications have been made according to the comments of the reviewer (page 5).

Reviewer: Leslie W Huson

Minor essential Revisions

1) As requested, we verified that the references of the figures match well with the text.

2) As suggested by the referee, we replaced "appropriate non parametric statistics tests" by "appropriate statistical tests" page 9.

Reviewer: Maxime Look

Minor essential Revisions

1) We agree that Anova is a parametric method. That is why, as mentioned above, we replaced "appropriate non parametric statistics tests" by "appropriate statistical tests" page 9.

2) As already discussed in our first correspondence, we fully agree with the reviewer that combining the present paper with another manuscript describing the technical and the clinical validation of NASBA for assessing uPA and PAI-1 mRNA could be of great interest in this field. That is why we are currently preparing such a manuscript summarizing data of technical comparisons between NASBA, RT-PCR and ELISA for uPA and PAI-1 and we hope to submit this manuscript in the next weeks.

3) As requested, "menopausal status" was replaced by "age categories" in the entire manuscript and in tables 3 and 4. We also added this parameter in table 1.

4) As requested previously, we added the hazard ratio for the univariate analysis in Table 3 and 4. As all the data described in this paper was based on "high" and "low" groups for uPA and PAI-1 gene expression level, we would propose for clarity reasons to keep both groups instead of continuous variables.

5) In the table 2, the tumor size was considered as a continuous variable since the true size of each tumor (in mm) was provided by the clinicians. Subsequently, we categorized the samples in T1, T2 and T3 groups according to standard clinical guidelines for univariate and multivariate analysis.

6) As for the tumor size, the exact number of lymph nodes was known. Thus, we considered the number of lymph nodes as continuous variables in the table 2 and thereby we used Spearman rank correlation test.

7) As discussed previously, the figure 1 was replaced by RT-PCR data. The scatter plot was removed.

8) We agree with the referee that the table 2 was a little bit confusing with regard to the explanations of the
different tests used. We thus modified the table 2 by labelling variables instead of values.

9) As requested by the reviewer, signs in descriptions of categories have been modified in Tables 1, 2, 3 and 4.

In addition, as requested by Iratxe Puebla, the senior assistant editor, we elaborated the Background section of the abstract to place the study in the context of the current knowledge in its field (page 2).

As requested, the figures were also cropped as closely as possible to minimise white space around the images. All figures in this article should be printed in black and white.

We hope that the manuscript is now acceptable for publication.

Sincerely yours,

Philippe Leissner, PhD