Author's response to reviews

Title: Celecoxib decreases prostaglandin E2 concentrations in nipple aspirate fluid from high risk women and women with breast cancer

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RE: Celecoxib Decreases Prostaglandin E2 Concentrations In Nipple Aspirate Fluid From High Risk Women And Women With Breast Cancer

Dear Sir:

Enclosed please find the revised manuscript which address each of the three reviewers' comments. Attached are the point by point responses to each concern.

Sincerely,

Edward R. Sauter

Reviewer 1
No required revisions

Reviewer 2
Major Compulsory Revisions
1. Use analytic methods that account for the lack of independence of observations... of affected and unaffected breasts in the same women.

Thank you for your thoughtful review. There were 9 women with cancer in one breast and not in the other for which NAF was collected and PGE2 analyzed. Tables 3 and 5, which analyze PGE2 values in plasma, only used the value once per subject, so these tables did not change. For Tables 2 and 4, NAF PGE2 values from the breast contralateral to that with cancer have been removed. This is reflected in revised Tables 2 and 4, with the new values and sample sizes in blue (in the text with reviewer revisions highlighted). In Materials and Methods, Specimen Collection subsection, we have revised the text to indicate that in all cases, NAF was analyzed from only one breast. If one breast had cancer and the other did not, NAF was analyzed from the breast with cancer, and serum values from these subjects were assigned to the cancer group.

In the previous and current version of the text, the Wilcoxon Rank Sum text was used for between group comparisons, and the Wilcoxon Signed Ranks test for within subject comparisons. This was stated incorrectly in the earlier version of the manuscript, which has now been corrected.

2. Use graphical methods appropriate to study design and cite figures in the results section.
We have created Figure 1 with two panels which address the changes in NAF in subjects that had a significant change in PGE2 after treatment. Figure 1A demonstrates changes in NAF from subjects with cancer, Figure 1B from high risk postmenopausal subjects. The Figure is cited in Results. We created Figure 2 which demonstrates changes in plasma from subjects with cancer. This Figure is cited in Results.

Regarding timing of specimen collection for the high risk vs cancer groups, all women had after treatment NAF and plasma collected within 12 hrs of their last dose of celecoxib, which is the half life of the medication. This has been added to Materials and Methods, Specimen Collection subsection.

3. Consider data transformation that could allow use of parametric methods...

Transformation of the data other than rank transformation was not necessary to answer the questions asked. As we note and you confirm, the goal of the current study was to motivate further, larger studies. In our view, sample sizes in the current data set are not sufficient for model building. However, a future study with sufficient sample size could be performed so that modeling could be done. In that case, considering additional transformations would be appropriate.

4. Explain discrepancies in numbers of women enrolled as stated in the methods and in the results sections.

We apologize for any confusion. Specifically, I believe you wanted an explanation for why there were 27 enrolled in Materials and methods, and 26+13=39 in results. 27 is the number recruited, both high risk and cancer, in 2004. 26 is the number of women at increased risk and 13 the number of women with cancer that were recruited during the entire study period, both in 2003 and 2004. Recruitment data have been moved to results. Reasons for ineligibility have been added. NAF success data has been moved to results.

5. Explain how subject compliance was assessed or monitored.

Compliance was assessed through the count of returned pills. All subjects were required to have taken at least 80% of the prescribed medication.

While the Wilcoxon rank sum test is appropriate for comparing pre vs postmenopausal groups, it is Not Appropriate for comparisons of pre vs posttreatment. Authors state they used Wilcoxon rank sum test for both situations. Authors also need to provide references for statistical tests and data analysis software used. Did the authors try data transformation that may have facilitated use of parametric methods?

6. Examine and present correlation, if any, of NAF and plasma PGE levels.

We examined before treatment, after treatment, and after washout correlations between NAF and plasma. We did this for the entire sample set, and then divided by menopausal group. There were no significant correlations found. This has been added to Results.

7. The lack of a placebo group also limits study findings, which the authors need to acknowledge.

This acknowledgement is now listed in the Conclusions.

Reviewer 3

Major revisions

1. What happened to recruitment data for 2003? What was the definition of evaluable and how many enrolled were unevaluable? What is a green top tube?

Thank you for your thoughtful review. We did not routinely keep data on those screened but not enrolled until mid 2003. All subjects enrolled were evaluable. Evaluable was defined as a subject in which we were able to collect a NAF or plasma sample and detect PGE2 before and after treatment. This has been added to the text. Text indicating green top has been revised to tube containing heparin.

2. Please clarify the statements in text regarding high risk women.

As the reviewer notes and the text states, in the high risk group, PGE2 decreased only in postmenopausal women. The comparisons in Table 4 that were significant were between pre and post treatment (before vs.
after), as well as pre vs washout. So the levels did significantly (p=0.02) decrease at the end of treatment. We agree that this is a preliminary observation which requires validation, as we state in the last sentence of the Abstract.

3. ...it is stated that a rise in NAF PGE2 levels was not seen after celecoxib treatment in contrast to the earlier study...

The text referred to a "nonsignificant rise in NAF PGE2..." in the earlier study. In the earlier study, the trend was in the whole group of high risk women. In the current study, the trend is only with premenopausal high risk women. In neither study was this trend significant. It is likely best not to discuss nonsignificant trends. As such, I have removed the text to which you refer.

Minor revisions
1. Decrease description of standard curve... for PGE2.
   This has been done.

2. Discussion should read endogenous levels of PGE2.
   This has been corrected.