Author’s response to reviews

Title: Preservation of Biomolecules in Breast Cancer Tissue by a Formalin-Free Histology System

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Version: 4 Date: 18 September 2007

Author’s response to reviews: see over
Dear Dr Saltman,

We are truly indebted to our reviewers for the guidance they provided in their thoughtful comments. We are submitting the revised manuscript and in the following we have addressed all of the reviewers concerns.

We modified the competing interest section as requested:

The authors have been granted one (M Nassiri, V Vincek, M Nadji) or more (AR Morales) patents for their inventions in histology. The University of Miami licensed these inventions which are the basis of UMFIX fixative (Tissue-Tek® Xpress™ Molecular Fixative), and Tissue-Tek® Xpress™ tissue processor to Sakura Finetek, Inc. The authors have received royalties and research support from Sakura Finetek. p10-Ln. 5.

Reviewer #1 Dr O’Leary

1- “On page 6, the authors …”
We have added the sentence as suggested: “We have not evaluated the suitability of HercepTest performed on tissues fixed in molecular fixative for predicting response to Herceptin” p7-Ln.6. Also we clarified it further in discussion section as “Clinical application of HercepTest performed on tissues fixed in molecular fixative need to be further studied in a larger series of cases and correlated with response to Herceptin” p9-Ln.18.

2- “At the bottom of the same phrase …”
We clarified this issue as: “These results indicate greater reverse-transcription efficiency and amplification in the UMFIX samples”. (p7 Ln.17) and in discussion
section as: “Better preservation of DNA and RNA was evident in our samples by greater amplification efficiency” p9-Ln.10.

Reviewer #2 Dr Liotta

1- We have followed the reviewer advice in providing the statistical analysis result in the abstract and the result sections for ER and HER2. We also omitted the all the subjective terms.

2- As our reviewer suggested previously we added FISH results to correlate it with IHC results. We added detail description in figure 2 legend to clarify it as:” Category (top) describes the result of ER IHC (0-3+), FISH for both UMFFIX and Formalin samples (Not Amp: not amplified, Amp: amplified), HER 2 IHC (0-3+). HER2 FISH result were the same in UFPE and FFPE sections, results only are shown for HER2 IHC 1-3+ cases. Each row represents one case.”

3- We have followed the ASCO/CAP guideline as standard in our interpretation of IHC and FISH studies, and stated it in manuscript:
In Methods section: “The HER2 reactions were scored according to ASCO/CAP recommendations [11]. A positive result for HER2 was considered when IHC staining of 3+ (uniform, intense membrane staining of >30% of invasive tumor cells) was present” p4-Ln 10.

“A fluorescent in situ hybridization (FISH) ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2 was considered positive or amplified; FISH ratio of less than 1.8 was considered negative or not amplified” p4-Ln 21.

4- We expanded the discussion of HER2 IHC studies in Discussion section:
“An often neglected pre-analytical step is the tissue fixation part. Lack of strict criteria in tissue handling steps has direct effect on therapeutic decisions that are being made based on tissue markers expression [10, 27]. Recent ASCO/CAP recommendation has addressed some of these steps [12]. For example, therapeutic decision is based on HER2 3+ positive results by IHC. The equivocal 2+ cases need to be confirmed by FISH. In our study 7 formalin-fixed samples had 2+ staining; three of these cases had 3+ score in parallel UMFFIX section and two
had 2+ score. All of the 3+ IHC positive cases by UMFIX were also positive by FISH. Therefore, there was no false positive result that might affect therapeutic decision making using UFPE tissue. Clinical application of HercepTest performed on tissues fixed in molecular fixative need to be further studied in a larger series of cases and correlated with response to Herceptin." p9-Ln 10.

I hope that our explanations are satisfactory.

I am looking forward to hearing from you.

Sincerely yours

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