Author's response to reviews

**Title:** Estrogen receptor alpha (ERα) mRNA copy numbers in immunohistochemically positive-, and negative breast cancer tissues

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**Author's response to reviews:** see over
To

The Editor
BMC Cancer

REF:
Manuscript # 1961808621131316
Title: Estrogen receptor alpha (ERα; mRNA copy numbers in immunohistochemically positive-, and negative breast cancer tissues
Authors: I. Poola and Q. Yue

Dear Editor,

Thank you for sending us the reviewer’s comments for our above manuscript. We are pleased to know that it will be considered for publication after a revision. We have received the comments by all the three reviewers and prepared the revised version taking all of their comments into consideration. The responses to each criticism are given below.

RESPONSE TO REVIEWERS COMMENTS

Dr. Slawomir Wolcznski

Dr. Wolcznski accepted the manuscript with out any revisions. We thank Dr. Wolcznski for his kind comments.

Dr. Jack Cuzick

This reviewer has suggested that the p values should be given to 2 significant figures and results and discussion should be in separate section with results confined to presenting data.

Response. We have given the p values to 2 significant figures (Page 9, last paragraph and Page 10 first paragraph) and separated the Results from Discussion sections. Discussion starts in Page 11.

Dr. Frederique Spyratos

Dr. Frederique agreed that QRT PCR is indeed a cost-effective, quantitative, sensitive and high throughput method but commented that implementation requires several modifications. In particular the reviewer has made the following comments. We have received those comments well and present here the following modifications/responses.

Criticism 1. Commented that we compared the results obtained in surgical samples but not in biopsies. The reviewer commented that the results obtained are not sufficient to validate for several reasons: Histological control of the sample analyzed is of major importance to ensure it is represented of the lesion and to evaluate the percentage of
cancer cells versus normal and stromal cells and it is not mentioned in the paper. Sample acquisition difficulties and histological control of the biopsies. IHC can be performed and it is done in a number of institutions.

**Response.** A number of samples we have analyzed were indeed from surgical, stereotactic and ultrasound guided biopsies. Tumor procurement and selection of tumor tissue for the ERα quantification were described in detail in our previous publications (Ref 6-9). Therefore, we did not reiterate the details here. However, in response to the above criticism we have included the details in the methods section (page 6, paragraph 2, lines 2 - 7).

**Criticism 2.** IHC and ligand binding assay may not correlate perfectly. The discordant results could be due to % tumor cells, menopausal status of the patient and eliminate the samples for which do not have quantitative data.

**Response.** Our QPCR data definitely showed that there is no difference in the mRNA levels in the samples that were positive by IHC and ligand binding assay. In the current study, our goal is to compare the ERα mRNA levels in tumors that are positive and negative by IHC and ligand binding assay procedures but not in tumors of different clinical parameters. Regarding the % tumor cells, response is addressed above.

**Criticism 3.** The reviewer commented that GAPDH cannot be used as a reference because its expression was shown to be associated with breast cancer cell proliferation and aggressiveness of tumors. GAPDH levels go down by chemotherapeutic drugs and bisphosphonates.

**Response.** GAPDH expression is widely used as a reference molecule in mRNA determination either by PCR or micro-array analyses. GAPDH expression is accepted as a reference for correlating the expression of several genes. Thus, our study is not an exception. Since all the samples we have used are cancer tissues (both ERα-positive and negative) that proliferate, we do not believe normalizing ERα mRNA levels to GAPDH has any flaw. Chemo or bisphosphonate therapy is given after diagnosis (removing the tumor by biopsy or mastectomy and testing for the presence of the cancer), and ERα could be assessed in the removed tumor tissue, therefore, treatments will not affect either the ERα or GAPDH quantification.

We hope the revised version is acceptable for publication. If you need any additional clarifications/modifications, please do not hesitate to contact me.

Thank you and best regards,

Sincerely,

Indira Poola, Ph.D
Professor of Biochemistry and Molecular Biology