Author's response to reviews

Title: Expression of estrogen receptor beta in the breast cancer cells of BRCA1 mutation carriers

Authors:

Maria M Litwiniuk (litwiniuk@skrzynka.pl)
Krzysztof K Roznowski (krzysztof.roznowski@oncology.am.poznan.pl)
Violetta V Filas (vfilas@wp.pl)
Dariusz D Godlewski (godlewski.open@wp.pl)
Malgorzata M Stawicka (gosiastawicka.open@wp.pl)
Remigiusz R Kaleta (remigiusz.kaleta@pharmacia.com)
Jan J Breborowicz (janbreborowicz@wp.pl)

Version: 2 Date: 26 January 2008

Author's response to reviews: see over
Dear Sirs

Enclosed is a final draft of the manuscript. It has been corrected and amended according to the suggestions of the four reviewers. A detailed list of the corrections and amendments is attached. It is addressed to each of the reviewers and the answers to their comments are provided point after point and reflect the order of the issues raised. I hope they will find this final draft satisfactory.

The paper has been once again read for language correctness as well.

Looking forward to hearing you,

Maria Litwiniuk

**Reviewer: Hiroko Yamashita**

**Major Compulsory Revision**

ad 1. certain additional information has been added as suggested. However, since the subject of the paper is focused on the expression of estrogen receptor, the authors feel that general description of the method should suffice.

ad 2. amended as suggested and the requested information has been added

ad 3. photographs of immunohistochemistry have been included as suggested

ad 4. Figure 1 has been removed as suggested

ad 5. information on the status in triple-negative breast cancer in the control group has been added

ad 6. out of the many research studies, the authors referred to only one (Gurvberger-Saal et al) because the authors were concerned that in a too long discussion the main idea of the paper might get lost

**Minor Essential Revisions**

All spelling and other typing errors have been corrected – the authors would like to apologize for overlooking those mistakes in some parts of the paper

**Reviewer: Dimitrios Stefanou**

Microscopic photographs have been included as requested.
Reviewer: Anna deFazio

As suggested, information on Daidone et al. study has been added

ad. 1. photomicrographs of immunohistochemistry have been included as suggested

ad. 2 appropriate corrections have been made

ad. 3 Yes, the antibody used to detect the receptor is able to detect both PR-A and PR-B. However, since the focus of the paper is on two estrogen receptors the authors feel that that particular information can be omitted.

ad.4. explanation on the status of patients with HER 2 = 2+ without amplification has been added as suggested

ad. 5 information on reagents has been added

ad. 6 for the sake of simplicity and clarity of the tables, it is believed that information on statistical tests may be omitted

ad. 7 as suggested, Fig.1 has been removed from the text

ad. 8 the terminology has been corrected and made consistent throughout the text

ad. 9 the lengthy discussion related to cDNA microarray studies results from the authors' fascination of the new technique as well as their observation that some of the basal like cancers may show the expression of ERβ

ad. 10 the abbreviation has been deleted as suggested

ad. 11 All spelling and other typing errors have been corrected – the authors would like to apologize for overlooking those mistakes in some parts of the paper

Reviewer: Abeer Shaaban

Major compulsory Revisions

The study presented in the paper was performed in 2003-2004. At that time there was only one antibody for ERβ available in Poland. This is a polyclonal rabbit antibody in which immunogen corresponds to NH2-terminus of the human ERβ, and, according to the manufacturer (Chemicon International www.chemicon.com), the sequence used is conserved in all known isoforms. Unfortunately, due to financial limitations (the work was carried out with no financial support) the authors did not ensure antibody specificity and relied only on the representation of the manufacturer.

A detailed method of immunohistochemical staining has been added as requested. Immunostained slides were evaluated by two independent observers in a "blinded" fashion – this information has been added.
The control group consisted of a number of subsequent cases occurring over the period of 1998-1999. In the authors' opinion, subsequent patients from a specified time range may be treated as a control group. As we all know, age influences ERα expression. However, as numerous studies have found, that is not true in the case of ERβ whose expression is not age-dependent.

As recommended, the authors briefly analysed the results using cut off value of ERβ positivity of 20% and the obtained results were found to be much the same as those quoted in the paper.

A list of BRCA1 associated cancer patients was obtained from the central register of heredity cancer patients treated in our hospital.

Regarding the control group - in the years 1998-1999 all breast cancer patients admitted to our hospital were offered genetic tests performed under a national cancer programme. About 70% of those had such tests performed. Consequently, it can be said that the majority of the control group patients were confirmed for the absence of BRCA1 mutations. However, since not all patients had the genetic tests performed, the name we gave to the group was a control group rather than sporadic. Having in mind though that among all breast cancer patients in Poland (unselected for age) those with BRCA1 mutations account for only 3%, the fact that not all of them had genetic tests performed cannot influence the overall result.

Photographs have been included as suggested.

Figure 1 has been removed according to the suggestion.

Minor Essential Revisions

All spelling and other typing errors have been corrected – the authors would like to apologize for overlooking those mistakes in some parts of the paper.

Most other suggestions and recommendations of the reviewer have been accounted for and parts of the text have been modified accordingly.