Author's response to reviews

Title: Immunohistochemical subtypes predict the clinical outcome in high-risk node-negative breast cancer patients treated with adjuvant FEC regimen: results of a single-center retrospective study

Authors:

Soraya Rahal (rahalsoraya@hotmail.com)
Jean-Marie Boher (boherim@ipc.unicancer.fr)
Jean-Marc Extra (extrajm@ipc.unicancer.fr)
Carole Tarpin (tarpinc@ipc.unicancer.fr)
Emmanuelle Charafe-Jauffret (jauffrete@ipc.unicancer.fr)
Eric Lambaudie (lambaudiee@ipc.unicancer.fr)
Renaud Sabatier (sabatierr@ipc.unicancer.fr)
Jeanne Thomassin-Piana (THOMASSINJ@ipc.unicancer.fr)
Agnes Tallet (TALLETA@ipc.unicancer.fr)
Michel Resbeut (resbeutm@ipc.unicancer.fr)
Gilles Houvenaeghel (HOUVENAEGHELG@ipc.unicancer.fr)
Lilian Laborde (labordel@ipc.unicancer.fr)
François Bertucci (bertuccif@ipc.unicancer.fr)
Patrice Viens (viensp@ipc.unicancer.fr)
Anthony Gonçalves (goncalvesa@ipc.unicancer.fr)

Version: 3

Date: 5 July 2015

Author's response to reviews: see over
Dear Editor-in-chief

Please find enclosed a revised version of our manuscript (MS:1495276931160406) “Immunohistochemical subtypes predict the clinical outcome in high-risk node-negative breast cancer patients treated with adjuvant FEC regimen: results of a single-center retrospective study” submitted for publication to BMC Cancer.

We would like to thank the reviewers for their positive and helpful comments. As you will see in the accompanying document, we have addressed all the points raised by the reviewers and made the requested revisions.

We hope that this improved version will meet your approval for publication in BMC Cancer.

Sincerely yours

Prof. Anthony Gonçalves
Medical Oncology Department
Institut Paoli-Calmettes
Centre de Recherche en Cancérologie de Marseille
INSERM U1068, CNRS U7258, Aix-Marseille Université
Marseille, France
Response to reviewer comments

A- Reviewer 1 (7728765271764641)

1. Major revisions

   a- The major limitation of the present manuscript is that the authors do not adequately discuss the limitations (and strengths) of the present study design to answer the research question. This was a retrospective (therefore non-randomized, subject to confounding and bias), single-centre, single group, cohort study. The authors did not discuss of the quality of the data sources (completeness/missing data, validation of the data) or possible bias and confounding factors (that may explain some of the results obtained). Since the present study design does not allow to establish causality, authors should be careful not to make strong causal statements. A final paragraph stating the overall implications of the study/future research could be added

   To take into account this comment (and also comments from Reviewer 2, see below), we have added in the discussion-section the following paragraphs (page 16, line 9):

   “This study has several strengths lying in the number of samples analyzed (more than 750), the duration of the follow-up (nearly 8 years in median) and the high-quality of data collected by a certified Data Management and Analysis Center. Weaknesses include its retrospective monocentric, non-randomized and non-comparative nature, the lack of central and ad hoc review of biological variables, the relatively high number of missing data for analysis of molecular subtypes (n=163), notably the number of missing HER2 status, the discussable approximation of luminal A/B distinction based on grade only, rather than on Ki67 and the low number of death events, precluding the identification of prognostic factors for OS. Another potential bias may be the definition of high-risk features used to indicate adjuvant chemotherapy during this period (pathological tumor size >15 mm, HR-negative tumors, SBR grade 2 or 3, PVI, or age <40), which is not the same as currently used. Finally, the relatively low number of HER2-positive patients receiving trastuzumab (49%) may also affect the clinical relevance of our results in routine practice.

   In spite of the above-mentioned limitations, our main result suggests that triple-negative and luminal-B/HER2-negative subtypes have a significant residual risk of relapse following FEC adjuvant chemotherapy. This may support the use of taxanes in these subsets of patients, even when node-negative, but additional researches are warranted in order to better predict a specific therapeutic benefit of this class of drugs according to the tumor features.”

   b- Other suggestions:

   Page 6, line 14: please clearly state what are the primary and secondary objectives/outcomes.

   To take into account this comment, we have completed the following paragraph mentioning our primary objective (page 6, line 10): “Our primary objective was to identify prognostic factors for DFS, which might help better defining node-negative BC patients candidate to the most aggressive
and expansive anthracyclines/taxanes combinations. Specifically, we have examined the prognostic impact of clinico-pathological parameters classically used to indicate adjuvant chemotherapy and that of immunohistochemically (IHC)-defined molecular subtypes of BC.” by adding (page 6, line 15): “Our secondary objectives were to describe OS and distant-disease free survival (DDFS) in this population. An additional secondary objective was to derive prognostic factors for DDFS.” This was also clearly stated again in the “Methods” section (sub-section: statistical analysis) by adding the following sentence (page 8, line 25): “The primary objective of this study was to identify prognostic factors for disease-free survival (DFS). Secondary objectives included description of distant DFS (DDFS) and overall survival (OS), as well as an exploratory analysis of prognostic factors for DDFS. Due to the low number of death events, no prognostic analysis was conducted for OS.”

Page 6, lines 18-19: the authors indicate that the data was collected from a prospectively maintained institutional database. I suggest describing the data source (the quality of the data used might be a strength that the authors did not mention in their discussion): is it an administrative or clinical database? Who collects the data? Is the data validated? Is there any missing data?

We have taken into account this comment by adding the following paragraph in the “Methods” section (page 9, line 21) : “The study population was identified from our prospectively maintained institutional database. This institutional clinical data base on breast cancer is operated by DATA MANAGEMENT AND ANALYSIS CENTER (DMAC), approved for its data management skills as data processing centers by INCa (French National Institute for Cancer) in 2007. DMAC is also ISO 9001 certified. Standard operating procedures are applied to collect, check and use data in the quality management system framework. Data are entered (source is medical records) in an ORACLE database by Clinical Research Assistant.”

Page 8, line 24: suggest STROBE guidelines instead

We have modified the sentence “We followed the reporting REcommendations for tumor MARKer prognostic studies (REMARK criteria) [14]” as follows (page 9, line 21): “We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [14]”

Page 12, line 12: OS is one of the secondary outcomes, but no results are presented

As mentioned above, the low number of death events prevented us from searching for independent prognostic factors for OS. Thus, OS analysis was only descriptive. This was mentioned in the “Methods” section (page 9, line 3): “Due to the low number of death events, no prognostic analysis was conducted for OS” and further discussed as a limitation in the “Discussion” section (page 16, line 12): “Weaknesses include its retrospective monocentric, non-randomized and non-comparative nature, the lack of central and ad hoc review of biological variables, the relatively high number of missing data for analysis of molecular subtypes (n=163), notably the number of missing HER2 status, the discussable approximation of luminal A/B distinction based on grade only, rather than on Ki67 and the low number of death events, precluding the identification of prognostic factors for OS.”

Page 13, lines 11 to 14 and Page 14, lines 19 to 21: how do the authors explain these results?

- Page 13, lines 11 to 14: this remark refers to the following sentence: “However, a sensitivity analysis focused on patients treated before 2005 September (date of initiation of adjuvant trastuzumab in
HER2-positive BC at our institution) did not show a significant adverse impact of HER2-overexpression in this population.” A potential explanation for this result was actually discussed in the following paragraph: “This may be related to the classically documented efficacy of anthracycline-based adjuvant chemotherapy in HR-negative tumors [1, 3]. In addition, a link between HER2 amplification and sensitivity to anthracycline-based regimen has been specifically suggested [22, 23], possibly due to frequent co-amplification or deletion of TOP2A, the gene encoding topoisomerase IIα protein, which is a supposed intracellular target for anthracyclines [24].”

To better discuss these results and enlighten our potential explanation, we have modified the structure of the whole paragraph as follows (page 13, line 17): “An explanation for the relative favorable outcome of patients with HER2 overexpression may be that a significant fraction of those patients received, after completion of chemotherapy and radiotherapy, adjuvant trastuzumab, the impact of which was largely demonstrated during the last 10 years [25, 26]. However, a sensitivity analysis focused on patients treated before 2005 September (date of initiation of adjuvant trastuzumab in HER2-positive BC at our institution) did not show a significant adverse impact of HER2-overexpression in this population. An alternative hypothesis could be the suspected link between HER2 amplification and sensitivity to anthracycline-based regimen [22, 23], possibly due to frequent co-amplification or deletion of TOP2A, the gene encoding topoisomerase IIα protein, which is a supposed intracellular target for anthracyclines [24].”

- Page 14, lines 19 to 21: this remark refers to the following sentence: “Notably and surprisingly, the luminal B/HER2-negative subtype was found to be even more aggressive, with a 5-year risk of relapse of 20%, including a risk of distant relapse of nearly 15%.” To further discuss these results, we have added the following sentence (page 15, line 10): “The aggressive clinical behavior of Luminal B tumors is well known and their prognosis has been considered as similar to that of HER2-enriched and basal-like groups[35]. However, while triple-negative and Luminal B/HER2-positive tumors might derive higher benefits from anthracyclines and/or anti-HER2 adjuvant treatment, some Luminal B/HER2-negative could be only sub-optimally treated with anthracycline-only regimen.”

Page 15, line 15 (page 17, line 4): In conclusion, our results suggest a relative...

This sentence was modified as required.

c- Minor essential revision:

Page 3, Title: should indicate that this is a retrospective study –

We have modified the title as required. It is now: “Immunohistochemical subtypes predict the clinical outcome in high-risk node-negative breast cancer patients treated with adjuvant FEC regimen: results of a single-center retrospective study”

Page 3, line 10: the term “Prospective data collection” is misleading

We have removed the term “Prospective”

Page 3, line 11: suggested modification “clinico-pathological characteristics and treatment information.”
We have modified this sentence as required.

**Page 3, line 12: SBR not previously defined**

This has been corrected

**Page 3, line 13: “were estimated” using the Kaplan-Meier Method?**

We have modified this sentence as required.

**Page 5, line 5: please review reference; study cited investigated the benefit of the addition of CMF to surgery**

The reference and the sentence have been modified: “Initially, polychemotherapy, notably cyclophosphamide-methotrexate-5FU (CMF) regimen, was shown to significantly decrease relapses and deaths over monochemotherapy or no post-operative treatment at all[2,3]”

**Page 5, line 13: please review reference, EBCTG 2005 showed this**

We have modified this sentence as required.

**Page 6, line 8: please define high-risk**

High-risk population has been defined in the “Method” section, as proposed by the reviewer (see below).

**Page 6, lines 8-9: “treated with adjuvant FEC chemotherapy”**

We have modified this sentence as required.

**Page 6, line 11: please clarify the meaning of “expansive” in this sentence**

We have replaced the term “expansive” by “costly”

**Page 6, lines 18 to 24: sentence is too long, suggest breaking and separating inclusion and exclusion criteria for the study.**

We have modified this sentence as required.

**Page 8, line 11: only median and ranges, counts and frequency were used**

We have removed the terms “mean, standard deviation”

**Page 8, line 12: suggest adding counts and frequency after categorical, using the term continuous instead of quantitative, and adding median and ranges after continuous.**

We have modified this sentence as proposed.

**Page 8, line 19: compared among immunohistochemical subtypes using the log-rank test?**

Since, univariate analysis included other survival comparisons than between immunohistochemical subtypes, we did not modify this sentence.
Page 10, line 22: “instead” not “in place” - We have modified it as required.

Page 11, line 1: “DDFS” not “DFS” - We have modified it as required.

d- Discretionary Revisions:

Page 5, line 24 and page 6 line 2: should be consistent with the terms anthracycline/taxane-based
We have modified it as proposed

Page 7, line 19 and 20: for consistency, please add “all grades”, after HER2-negative and after HER2-positive
We have modified it as proposed

Page 8, line 1: can explain here that these are the high-risk features
We modified the sentence as follows: “Adjuvant chemotherapy was indicated for pN0 patients in case of high-risk features, defined as: pathological tumor size >15 mm, HR-negative tumors, SBR grade 2 or 3, PVI, or age <40.

Page 10, line 1: suggest using the term clinico-pathological predictors of DFS
We have modified it as proposed

Page 10, line 2: In the univariate analysis (and every time the term is used after this)
We have modified it as proposed

Page 10, line 8: In the multivariate / adjusted analysis (and every time the term is used after this)
We have modified it as proposed

Page 10, line 12-13: suggest “were independently associated with an adverse outcome” instead
We have modified it as proposed

Page 10, line 18: suggest using the terms predictive or associated with instead of prognostic
We have modified it as proposed

Page 11, line 21: suggesting the these patients might...
We have modified it as proposed

Page 23, Table 2: suggest removing the 5-year DFS column from the table. The same for page 25, table 4.

We believe that these data could be useful for readers, especially in case of comparison with their own data. Accordingly, we have conserved these columns.
B- Reviewer 2 (1613024962166215)

1. **Major revisions**
   - *I suggest to the authors a better adherence to STROBE guidelines (von Elm et al, PLOS Medicine 2007) for reporting results of an observational study such as for example the introduction of a flow diagram with a clear indication of all patients excluded by analysis and relative reasons;*

   As proposed by the Reviewer 2, we have introduced a flow diagram which details the different steps of population analysis (Figure 1). In addition, adherence to STROBE guideline was checked and a clear reference to STROBE guidelines was added in the “Methods” section.

   - *I suggest to the authors clearly state the limitations of study in the discussion such as the retrospective nature of the study, the high number of not available molecular subtypes and the lack of a central and ad hoc review of the biological variables.*

   The limitations of the study have been clearly stated by adding the following paragraph in the “Discussion” section (page 16, line 9):

   “This study has several strengths lying in the number of samples analyzed (more than 750), the duration of the follow-up (nearly 8 years in median) and the high-quality of data collected by a certified Data Management and Analysis Center. Weaknesses include its retrospective monocentric, non-randomized and non-comparative nature, the lack of central and ad hoc review of biological variables, the relatively high number of missing data for analysis of molecular subtypes (n=163), notably the number of missing HER2 status, the discussable approximation of luminal A/B distinction based on grade only, rather than on Ki67 and the low number of death events, precluding the identification of prognostic factors for OS. Another potential bias may be the definition of high-risk features used to indicate adjuvant chemotherapy during this period (pathological tumor size ≥15 mm, HR-negative tumors, SBR grade 2 or 3, PVI, or age <40), which is not the same as currently used. Finally, the relatively low number of HER2-positive patients receiving trastuzumab (49%) may also affect the clinical relevance of our results in routine practice. “

2. **Minor revisions**

1) pag 3 line 9: epirubicin instead of epirubicine

   We have modified it as proposed