Author's response to reviews

Title: Frequency, prognostic impact, and subtype association of 8p12, 8q24, 11q13, 12p13, 17q12, and 20q13 amplifications in breast cancers

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Version: 2 Date: 22 September 2006

Author's response to reviews:

Dear Editor,

Thank you very much for the review process of our manuscript MS ndegrees 5383020001115572. We are very grateful to the reviewers for their helpful comments. We found all of them relevant. We have answered the comments as follows:

Reviewer one (Peter Schraml)
General comment:
Many of the data have indeed been published in various studies but never in a single one. A most comprehensive study on five regions of amplification in breast cancer has been reported by Al-Kuraya and colleagues in 2004. We have here studied six regions. Only three are common between the two studies. In addition we report on a new amplification, at region 12p13 (as mentioned by the expert himself below) and its association with the basal subtype.

Major comments:
- Comment 1 (this also concerns comment 1 from reviewer 2). The same comment from the two experts makes it an important issue. The rationale to break up regions of amplification in subregions is that they are often made of several distinct subamplicons. The prognosis associated with the amplification of these subamplicons may be different. We have recently shown this for the 20q13 amplification (Ginestier et al., Clin Cancer Res 2006) and the 8p12 region (Gelsi-Boyer et al., Molecular Cancer Res 2005). However, we agree with the reviewers that this made the manuscript very difficult to read. To follow their recommendations we have simplified the manuscript: we now consider only one set of amplifications (the former group II) instead of two groups.
- Comment 2: To answer this comment, which is similar to comment 3 of reviewer two, we have added a new figure showing the various amplification tested with the corresponding probes, and the respective frequency of each amplification. This is new Figure 1. Since we have removed the former Figure one, the total number of illustrations has not changed.
- Comment 3: "This study would gain more importance if the amplification profiles and frequencies of each of the genes residing on 8p11-12 and 20q13.1.3 are shown and discussed in more detail". A main topic of the study focused on the evaluation of the clinical impact of six amplified regions on outcome and other clinical features. To rigorously answer this comment, it is important to know that for only the 8p12 region, the number of genes spanned by BAC pools would need to use other approaches than FISH analysis, such as QPCR or high density array-CGH. Although this question is very important too, this does not correspond to the topic we wanted to underline in this article.
Concerning NOL1 amplification, we integrated information throughout the main text p16.
- Comment 4: The reason for so few samples being informative for all FISH is rather simple. It has to do with the TMA itself and not only with the FISH. The TMA we used begins to be exhausted and several cores were missing. This is commented in the text /Result section (Frequencies of amplifications and co-amplifications).

Minor comments:
We corrected the manuscript. We modified the title. We checked the references. We added the method to obtain survival data in the relevant section. We modified Figs 1 and 2.
The term "intact" was not appropriate. We replaced it by "no amplification".
Concerning the network figure (Figure 2), more explanations are given throughout an example in the corresponding legend.

Reviewer two (Larissa Savelyeva)

Major comments
- Comment 1: same as reviewer 1.
- Comment 2: 100% corresponds to the number of informative cases found for each analyzed region. This information is given in the table of the new Figure 1.
- Comment 3: see comment two of reviewer one, and the new figure 1. We now consider only one set of amplifications (the former group II) instead of two groups. To facilitate the reading of this article, we have followed the suggestion of this reviewer in drawing schematic representation of amplified regions tested with corresponding FISH probes in the new Figure 1.
- Comment 4:
a - "number of the tumors analyzed" corresponds to the number of informative cases found for each analyzed region. This information is given in the table of the new Figure 1.
b - short notes below the tables were added for Supplementary Table 1 and 2. (ST1 and ST2). In the Supplementary Table 2, the sum of the number of tumors analyzed for the same region can be different because some tumors are not informed for all clinical features.

Minor comments
1-Web resource of R.2.3.0 statistical software was mentioned in the text p10.
2-9.9% is the correct value which was presented in the similar way.
3- Two references were added in the text:
Cuny et al., 2000 and Al-Kuraya et al., 2004: p14
4-The reviewer is right; we corrected accordingly: p16
"Amplification of the 11q13 region is a relatively frequent event in breast tumors..."
In the context, the reference Struski et al. was indeed not relevant and was removed and replaced by Cuny et al., 2000 (ref#3)
We have corrected the manuscript to answer to these comments.

We hope that the reviewers will be satisfied by the corrections. Thank you again for giving us the opportunity to improve our manuscript.

Sincerely,

Max Chaffanet