Author's response to reviews

Title: Unraveling the chromosome 17 patterns of FISH in interphase nuclei: an in-depth analysis of the HER2 amplicon and chromosome 17 centromere by karyotyping, FISH and M-FISH in breast cancer cells

Authors:

Milena Rondon-Lagos (rondon.sandra@ur.edu.co)
Ludovica Verdun di Cantogno (lverdundicantogno@cittadellasalute.to.it)
Nelson Rangel (rnelsone@gmail.com)
Sandra R. Ramírez-Clavijo (sandra.ramirez@urosario.edu.co)
Teresa Mele (teremele@gmail.com)
Giorgio Scagliotti (giorgio.scagliotti@unito.it)
Caterina Marchiò (caterina.marchio@unito.it)
Anna Sapino (anna.sapino@unito.it)

Version: 4 Date: 16 November 2014

Author's response to reviews:

Caterina Marchiò
Anna Sapino
Via Santena 7
Turin, 10126
+390116335464
+390116635267
caterina.marchio@unito.it
anna.sapino@unito.it

Turin, November 15th 2014
To Dr. M. Brunelli
Editor of BMC Cancer

Dear Sirs,
Following the reviewers' comments we are now resubmitting a revised version of the paper entitled “Unraveling the chromosome 17 patterns of FISH in interphase nuclei: an in-depth analysis of the HER2 amplicon and chromosome 17 centromere by karyotyping, FISH and M-FISH in breast cancer cells” to BMC Cancer.

We have taken into account the suggestions made by both reviewers as detailed here below.
Reviewer#1:
The Authors evaluated chr17 rearrangements in metaphases of 9 breast cancer cell lines and a primary culture from a triple negative breast carcinoma by using G-banding, FISH and M-FISH. They found high frequency of complex chr17 abnormalities, such as losses, gains and break-riarrangements. The study is of interest and innovative.

Minor:
#the characterization of triple negative breast carcinoma has been performed on a single case, thus the conclusion of the key cytogenetic features of chr17 in this subtypes of breast cancer must be chary. Insert a statement in which the Authors explain the heterogeneity of triple negative breast carcinoma and conclude that these features have to be confirmed on further cases.

AUTHORS: we agree with the reviewer that the analysis was limited by the only sample analyzed. The primary cell lines was of help for us to make a parallelism between immortalized cell lines (that are subjected to many passages and can accumulate chromosomal alterations and may not be representative) and tumors derived from a patient: the fact that chr17 was altered also in this primary cell line corroborated our hypothesis that chromosomal alterations involving chr17 in breast cancer may be indeed very complex, however we acknowledge that it must be clarified that these results cannot be generalized to triple negative carcinomas. Therefore we have amended the text in the discussion.

#monosomy of chromosome 17 has been reported in a subset ranging from 1% to 30% of breast carcinomas. Do the Authors interpret any cases be characterized by monosomy of chromosome 17 after the analytical phase showing losses of more loci? Did any techniques used in the study reveal any interpretation of monosomy of chr17? If not, please insert few rows in the Discussion making a statement on monosomy of chromosome 17.

AUTHORS: we agree that the topic of monosomy is of great interest and it would be interesting to have data from in vitro cell lines to provide direct evidence of monosomy of chromosome 17. In this study we did not identify monosomy of chromosome 17 by M-FISH painting in any of the breast cancer cell lines analyzed. We cannot rule out that a larger collection of breast cancer cells (especially primary cell lines) would allow to observe such a phenomenon. This was not a technical issue or a feature connected to the polyploidy proper of these immortalized cell lines, as when we looked for monosomy in any of the chromosomes we found monosomy in some of the chromosomes (see table in the attached file).

Regardless of our findings, we agree with the reviewer that this topic is of great interest and added a comment in the discussion.

#the study has been well conducted, however my view is that the conclusion proposed from the study is not the most appropriate: 1) the several pattern of abnormalities revealed from the study pose the attention to the design of new clinical trials in which the subtypes of chromosomal complexity of chr17 may
explain, at least in part, resistance to targeted therapies or scheme of chemiotherapies. Again, the complexity of abnormalities may help correlation with clinical end-points, such as prognosis when focusing on DFS or OS; 2) the ASCO/CAP 2013 guidelines well described the difference between the analytical and interpretative phases and they updated the rules to interpret at best the differences of counting the locus specific probes versus the centromeric ones, visible during the analytical phase. I believe the conclusion is not avoid counting chr17, is to improve guidelines of interpretation. Differently, if we use only the single Her-2/neu probe we could lose many information that might be of interest at clinical level. Therefore, my suggestion is to modify the conclusion in the Abstract and at the bottom of the manuscript. The complex abnormalities of chr17 merit further investigation at clinical level, being potentially in correlation with responsiveness or resistance to targeted or personalized scheme of therapies. I agree with the Authors, in addition to the aforementioned statements the cytogenetic complexity of chr17 must be taken in account when consensus guidelines are encountered.

AUTHORS: we agree with the reviewer that this scenario of complexity of Chr17 may indeed take part in determining or participating to the resistance to targeted therapies. The complex abnormalities of Chr17 are definitely worth pursuing further investigation at clinical level, being potentially in correlation with responsiveness or resistance to targeted or personalized scheme of therapies. As an example, it has been recently reported that CEP17 duplications correlates with higher pCR rates than HER2 and TOP2A do. According to the reviewer’s suggestion we have modified the conclusions in the abstract and in the manuscript. We have therefore added a comment about this topic in the discussion and quoted these additional findings recently published in Neoplasia this year.

when dealing with external and internal quality control assessments on molecular Her-2/neu testing, main focus is posed on the problematic located at the pre-analytical phase. Your findings again reflex the fact that some discordances in between immunoistochemical and cytogenetic analysis may be due (in part) to biological reasons rather than pre-analytical problematics. Insert a statement in the Discussion focusing on this problematic.

AUTHORS: we agree with the reviewer that due to the complex chromosomal rearrangements we may have a very complex scenario in some cases and with only immunohistochemistry and in situ hybridization in interphase nuclei we may just see a partial picture. We have therefore added a comment in the discussion section.

Tables. Insert a column or an small insert into the images, showing Her-2 testing as performed by blinded dual color Her-2/CEP17 probes and as performed by blinded single Her-2 probe, showing if discordances are observed per each of the 10 cases.

AUTHORS: given that no differences in final results of HER2 gene status were observed by using the dual signal or the single signal assay method in the cell lines analyzed (whenever there was a gain of CEP17 the cell lines here analyzed
always showed clusters of HER2 signals that allowed to label the cells as positive for HER2 amplification) we decided to include this information in the legend of table 3.

#do the Authors have chances to perform immunophenotypical analysis on 10 breast carcinomas?

AUTHORS: unfortunately we do not have the chance to include tumor samples in the present paper and analyzed them by comparing IHC, FISH, M-FISH. The only primary culture included in the paper was of triple negative phenotype (HER2 score 0 by IHC).

Reviewer#2:
Line 64
‘normal’ -> non-altered copies of Ch17; as this cell line contained polysomic Ch17 presence
Lines 68, 69
Amend the sentence to read: ‘only CEP17 signals were detectable in the rearranged or altered positions’
Line 77
Amend the sentence to read: ‘not always indicative of normal unaltered or rearranged copies of Ch17’
Line 115:
Amend the sentence to read: ‘about 10% of all IHC score 2+..’

Results
To emphasise the most notable findings in bold type
Line: 255
‘Notably, 8 of the’
Line: 256
‘39 rearranged chromosomes carried CEP17 signals without HER2 and STARD3 signals’
Line: 257
‘and 14 harbored HER2 and STARD3 genes but not CEP17.’
Line 286
MCF7 interphase nuclei displayed four CEP17 green signals and two red signals for the HER2 and STARD3 genes (Table 3, Figure 6). This pattern corresponded to
Line288
one CEP17 signal and one copy of the HER2 and STARD3 genes located on two
normal Chr17 and two CEP17 signals on two Chr17 derivatives as confirmed by M-290 FISH (Figure 1). The FISH pattern for TOP2A was similar to that observed for the HER2 and STARD3 genes, with the only exception of having an additional TOP2A copy mapping on a derivative chromosome 6 (Figure 6 and Figure 1).

AUTHORS:
We thank the reviewer for the careful revision of the paper and we have now amended the text accordingly.

Altogether, we feel that the criticisms raised by the reviewers helped improve the paper and we now hope that this version of the manuscript may be deemed suitable for publication in BMC Cancer. All changes have been highlighted in blue color in the revised version of the manuscript.

Should you have any queries please do not hesitate to contact us.

Sincerely, Caterina Marchio and Anna Sapino