Author's response to reviews

Title: No significant role for beta tubulin mutations and mismatch repair defects in ovarian cancer resistance to paclitaxel/cisplatin

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Author's response to reviews: see over
Dear Editor-in-Chief Dr. Peter Newmark

Thank you very much for the comments on our manuscript “No significant role for beta tubulin mutations and mismatch repair defects in ovarian cancer resistance to paclitaxel/cisplatin” by Mesquita et al. We have revised the manuscript in light of the reviewers’ comments as follows:

Reviewer: Michael Kelley

**Major compulsory revision 1: MSI analysis**

We agree with the reviewer that the MSI analysis with five markers would be more detailed than with two markers, but our study was retrospective and it was not possible to obtain normal DNA from the patients to match with tumour DNA (we have used archival tumor tissue embedded in paraffin). To circumvent this problem, we decided to use the MSI markers BAT 26 and BAT 34 because there is extensive evidence in the literature showing that this is a quite informative alternative to determine the MSI status of a tumor:

1. Hoang et al (1997) [Hoang JM, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R. BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. Cancer Res 1997; 57: 300-3] demonstrated that BAT 26, a mononucleotide marker with a quasimonomorphic profile in populations studied to date, presented a 99.4% of efficiency to detect MSI status.


3. The same group [Zhou XP, Hoang JM, Cottu P, Thomas G, Hamelin R. Allelic profiles of mononucleotide repeat microsatellites in control individuals and in colorectal tumors with and without replication errors. Oncogene 1997; 15: 1713-8] also showed that BAT 34 and BAT 25 were the best choices after BAT 26, because they were also quasimonomorphic in normal DNA and MSI stable tumours and presented shortened alleles in RER+ colorectal cancers.

4. That this is a quite informative approach is also stated in the international criteria for MSI analysis [Boland RC, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58: 5248-57].
Taken these data together, we feel confident that our analysis with these two quite informative markers allows us to conclude that MSI does not play a significant role in ovarian cancer chemoresistance.

**Major compulsory revision 2: Single trace for each STR marker**

As shown above, MSI analysis using quasimonomorphomic markers like BAT 26 and BAT 34 does not involve comparison with normal DNA from the same patient. The single trace we show in Figure 3 for each marker is the normal pattern found in every tumor and in every normal control DNA sample we tested.

**Minor essential revision 1: Description of the microdissection technique**

We have now included a more detailed description of the technique used for tissue dissection under the sub-heading “DNA extraction”. The minimum percent of tumor cells obtained by this technique was 70%, a proportion that is well suitable for mutation detection by sequence analysis.

**Minor essential revision 2: Amplification of pseudogenes**

In order to avoid co-amplification of pseudogenes, we have used an intronic primer set for the first round PCR. Nested PCR was performed after checking for first round PCR products in an agarose gel and the second PCR was performed after a 1:10 dilution. This strategy, which is now made more clear under the sub-heading “TUBB exon 4 sequencing”, makes significant amplification of pseudogenes very unlikely.

**Minor essential revision 3: GenBank accession number**

We have mistakenly written GenBank accession number J00314 in the manuscript, but we have used the correct GenBank sequence accession number AF070600. This has now been corrected.

**Discretionary revision 1: Figure 1**

We which to keep figure 1 because it helps to show that the amplification products obtained are from beta tubulin exon 4 and not pseudogenes. Since this is an online journal, there are no major space limitations.

**Reviewer:** Rafael Rosell

**Points 1 and 3: More detailed results**

We have provided a more detailed description of the results, both in the abstract and in the Results section.

**Points 2 and 6: Evaluation of response**

The evaluation of clinical response to chemotherapy was done by an oncologist based on computerized tomography or magnetic resonance and CA125 quantification,
according to international guidelines. This is now clear under the sub-heading “Patient data”. The expression “results were not matched until the study was completed” is now substituted by “Investigators performing laboratory analysis were not aware of chemotherapy response or resistance until the study was completed”.

**Point 4: Reference to cell lines and use of commas**

The expression “in hamster cells and in ovarian cancer cell lines”, in the second paragraph of Discussion, is now changed to “in ovarian cancer cell lines and in hamster cells”. Commas are now changed to periods.

**Point 5: Grammar**

We have revised the manuscript to avoid expressions like “on the other hand” and “ab initio”.

**Point 7: Figure legends**

We have now more complete information in figure legends.

The manuscript has also been revised by colleagues proficient in English. Hoping you now find our manuscript suitable for publication in *BMC* Cancer, I send you my best regards.

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