Author’s response to reviews

Title: Genomic profiling in ovarian cancer retreated with platinum based chemotherapy presented homologous recombination deficiency and copy number imbalances of CCNE1 and RB1 genes

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To
Dr Christina M Annunziata
BMC Cancer
Dear Dr Annunziata,

We would like to thank the reviewers for the constructive criticism regarding our manuscript entitled “Genomic profiling in ovarian cancer retreated with platinum-based chemotherapy presented homologous recombination deficiency and copy number imbalances of CCNE1 and RB1 genes”

In this revised version, the alterations are highlighted in grey. We have also attached our response and comments point-by-point, as recommended.

All authors have read and approved the revised version of this manuscript.

We look forward to hearing from you concerning the suitability of the revised manuscript for publication.

Yours sincerely,

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REVIEWER 1 (Kirsten Timms, PhD)

The manuscript describes an analysis of several previously described biomarkers for response to DNA damaging agents in a small cohort of platinum resistant ovarian cancer. While the cohort is small and its size limits the conclusions that can reliably be drawn, the content of the study is of interest due to ongoing clinical trials of PARP inhibitors in this treatment setting. The following concerns should be addressed by the authors:

1. The methods which describe the analysis performed and algorithms utilized in order to generate the 3 HRD scores described in the manuscript need to be more comprehensive.
Currently the authors provide only a reference to a previously published manuscript (Telli et al., 2016), however the assay described in that manuscript (next-gen sequencing based) is substantially different from the assay used in the current study (SNP microarray). Given that the authors have utilized thresholds developed by Telli et al. for use with their assay it is important to understand whether HRD scores generated by the two assays are comparable. Any variation in scores between the two methods would result in false negative or false positive samples within the current study. The description of the analyses performed in order to calculate the HRD scores are not comprehensive enough for a reader to be able to assess whether this is the case.

ANSWER: In fact, only the CS score described in our manuscript is based on Telli et al. [1], which is derived from Next Generation Sequencing. The thresholds used for LOH [2] and LST [3], are both derived from SNP microarray. As recommended, we presented a detailed explanation of the HRD scores used in the Methods section (page 9), as follows:

“LOH was calculated as the number of LOH regions spanning at least 15 MB but not involving the entire chromosome. The number of regions with allelic imbalance extending to one of the telomeres but not crossing the centromere, after filtering regions shorter than 11 MB or spanning less than 500 probes, was defined as tAI. The LST score was defined as the number of breakpoints between regions spanning at least 10 MB within a distance of maximum 3 MB. HRD was computed as LOH + tAI + LSTm (adjusted LST score). According to Timms et al. [26], the HRD score introduced by Telli et al. [25] increases with ploidy in both intact and deficient samples. The adjusted LST score is calculated as [LSTm=LST-15.5P], in which P is the tumor ploidy. Using logistic regression analysis, the constant 15.5 was derived to provide the best separation between intact and deficient samples. ASCAT [27] was used for inferring tumor ploidy, calculating allele-specific copy numbers and segmentation.”

References:


2.1- One interesting observation from the current manuscript is the high rate of CCNE1 amplification in this cohort, and the overlap of CCNE1 amplification with BRCA1/2 mutations. Previous studies have reported CCNE1 amplification at lower rates.

ANSWER: We found a frequency of CCNE1 gains (defined as copy number > 2) in 9 of 15 (60%) tested tumors. Most studies reported a frequency of 20-30% of CCNE1 amplifications [1-4]. In our opinion, the main reason for this difference is the highly selected population that we tested. In our study, all cases had platinum resistant recurrences while other published cohorts were composed by patients with ovarian cancer in their primary treatment. As pointed out in the Discussion section (page 16), 10 of 15 tested patients presented primary platinum resistant recurrences, and 7 of 9 patients with CCNE1 gains were among those with primary platinum resistant disease. The frequency of CCNE1 gains higher than expected may be related to the specific characteristics of the population studied herein.

We added the following sentence in the Discussion section (page 16): “This finding supports the association of CCNE1 aberrations and resistance to platinum therapy and may explain the higher frequency of CCNE1 gains observed in our study.”

References:


4- Stronach EA, Paul 2, Timms KM, Hughes E, Brown K, Neff C et al. Biomarker Assessment of HR Deficiency, Tumor BRCA1/2 Mutations, and CCNE1 Copy Number in Ovarian Cancer: Associations with Clinical Outcome Following Platinum Monotherapy. Mol Cancer Res. 2018 Jul;16(7):1103-1111
and that CCNE1 amplification and BRCA1/2 mutations are mutually exclusive (Cancer Genome Atlas Network, 2011; Ciriello et al., 2012; Etemadmoghadam et al., 2013). In a recent publication (Stronach et al., Mol Cancer Res, 2018) analysis of HRD scores, BRCA1/2 mutation status, and CCNE1 amplification in a cohort of 250 ovarian tumors reported that CCNE1 amplification and BRCA1/2 mutants were again mutually exclusive, and that there was a highly significant association between CCNE1 amplification and low HRD scores (<42). The authors of the current study should discuss possible reasons why the previously reported relationship between CCNE1 amplification and BRCA1/2 mutation status does not appear to hold true in this cohort, and in addition should provide an analysis of the relationship between CCNE1 amplification and the HRD scores which they have calculated.

ANSWER: Two of four patients with BRCA variants presented CCNE1 gains. These two patients had 2.2 and 2.3 CCNE1 copy numbers (as shown in our table 2). The definition of CCNE1 amplification is different according to each study. We used the term “gain” to express a copy number > 2. Stronach et al. used the value of 2.4 using the copy number detected by SNP array [1], while Aziz et al. used the ratio 19q12: INSR ratio ≥ 3 detected by FISH to define CCNE1 amplification and called a ratio between 2.0 and 2.9 as low copy number gains [2]. A recent study (phase II trial of CHEK1 inhibitor) using Taqman® MGB probes defined amplification as a copy number > 3 and copy number gain for values between 2.1 and 3.0 [3]. Using these aforementioned studies, none of our two BRCA mutated patients would be considered to have CCNE1 amplification.

Moreover, even in the studies that showed BRCA mutations and CCNE1 amplifications mutually exclusive, few cases of BRCA mutated tumors also harbored CCNE1 amplifications. Ciriello et al. (2012) using the TCGA data detected at least four patients with BRCA mutations and CCNE1 amplification [4]. Aziz et al. (2018) reported at least six patients with BRCA mutations and CCNE1 amplification [2]. Etemadmoghadam et al. (2013), assessing copy number by quantitative PCR, described that CCNE1 gains and BRCA mutations were mutually exclusive only in the presence of high level CCNE1 amplification and BRCA mutation [5].

The co-occurrence of CCNE1 gains and BRCA mutations described in our study could be explained by the low level gains in these two cases. The same holds true for the association between HRD and CCNE1 gains (showed in table 2 of the manuscript). Three of 15 patients showed HRD biomarker positivity and CCNE1 gains. Two of them had low copy number gains (2.1 and 2.2), while one presented a copy number of 2.6.

In the Results section - Genomic alterations (page 13), we added the following sentence: “Five of eight patients with CCNE1 gains presented low HRD scores (Table 2).”

Additionally, we modified the Discussion section (page 16), as follows: “Previous studies showed CCNE1 gains and BRCA mutation or homologous recombination deficiency as mutually exclusive [29]. However, the authors showed that complete mutually exclusive alterations were
not observed between low levels of CCNE1 gains and BRCA mutations. Our two patients with co-occurrence of BRCA mutation and CCNE1 gain presented low CCNE1 copy number gains (2.2 and 2.3).”

References:

1- Stronach EA, Paul 2, Timms KM, Hughes E, Brown K, Neff C et al. Biomarker Assessment of HR Deficiency, Tumor BRCA1/2 Mutations, and CCNE1 Copy Number in Ovarian Cancer: Associations with Clinical Outcome Following Platinum Monotherapy. Mol Cancer Res. 2018 Jul;16(7):1103-1111


REVIEWER 2 (Christina M Annunziata, MD, PhD)

Summary: This study presents genetic analysis of 31 ovarian cancer tumors from Brazil. The authors measured chromosomal copy number and analysis of a selected panel of mutations. Aberrations are linked to clinical data especially response to platinum chemotherapy.

Comments:

1. Please comment on the BRCA mutations listed in Table 2, as to whether they are known deleterious mutations.
ANSWER: According to ClinVar, the BRCA1 c.3931_3934delAACA is a known pathogenic variant caused by a deletion of four bases that leads to a frameshift variant. Two variants presented conflicting results (BRCA2 c.8350C>T and BRCA1 c.5096G>A) in the ClinVar. We added an additional table (additional file 4) including the information of each BRCA variant according to ACMG - American College of Medical Genetics and Genomics (ACMG). The additional table is cited on page 12 as follow: “Detailed information on BRCA1 and BRCA2 mutations are presented in additional file 4.”

2. It is also unusual to have a BRCA mutation in the absence of p53 mutation - it may be worth emphasizing whether these are somatic mutations since they were sequenced from tumor.

ANSWER: The mutational profiling of all cases of our study was obtained by tNGS performed in tumor samples and the allelic frequency detected for the genes tested is compatible with somatic mutations. We added this sentence in the Results section – Genomic alterations on page 12. Two of four patients with BRCA mutations have no TP53 mutations, including one undifferentiated carcinoma and one high grade serous carcinoma. High grade serous carcinomas are expected to harbor TP53 mutations in 96% of cases according to TCGA [1]. In high grade serous carcinoma, BRCA mutations are expected to occur without TP53 mutations in only 1.4% of cases [2]. Although BRCA mutations are more frequent in high grade serous carcinoma, it has been shown that other histological subtypes such as low grade serous, clear cell and high-grade endometrial cancers may present BRCA mutations in a lower frequency [3]. These subtypes have a much lower frequency of TP53 mutations. A plausible explanation for our findings is that our cases are among the rare high-grade serous carcinoma with BRCA mutation and absence of TP53 mutation.

References:


3. Many of these findings have been published previously. Please comment in the discussion on how this study adds new knowledge to the literature.
ANSWER: To better clarify the relevance of our study, we added the following paragraph in the Discussion section (page 18):

“In this study we explored the mutational profile and HR deficiency score in ovarian cancer patients to better understand the platinum resistant recurrence as defined by the platinum free interval. We demonstrated that HR deficiency scores, CCNE1 gains and RB1 losses could be used to distinguish patients who are still sensitive to platinum retreatment from those resistant to platinum therapy. Considering similar mechanisms of sensitivity to platinum salts and PARP inhibitors, these markers could be useful to better select the patients for PARP inhibitors therapy in the platinum resistant relapse.”