Author's response to reviews

Title: Cancer-testis antigen Cyclin A1 is broadly expressed in ovarian cancer and is associated with prolonged time to tumor progression after platinum-based therapy

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Author's response to reviews: see over
Dear editor,

We appreciate the reviewers’ careful reading and thoughtful comments on the previous draft of our manuscript. We carefully considered their comments in preparing our revision and addressed each reviewer’s concerns as outlined below.

We felt that we had to make new figures for Fig 1, 2, and supplemental Fig 2. Unfortunately, we discovered that we had made a mistake concerning the threshold line in Figure 2 (which was supposed to be mean+3*SD=0.23) and the numbers of samples. When we initially identified the samples, which were ‘positive’ for Cyclin A1 as we defined it in our method section, we discovered that we had used 2.3 instead of 0.23 as a threshold. Furthermore, as you can see in the initially submitted figure, the number of samples is 9, not 8. And because of the lower threshold, not 5 out of 8 samples but 7 out of 9 samples have to be declared ‘positive’. We now corrected this mistake thorough the manuscript.

Responses to Reviewers’ Comments

Responses to Reviewer #1 (yellow color)

Results

Comment: 1) Lines 229-231: “…at least moderate Cyclin A1 expression in (Cyclin A1 high) was associated with prolonged TTP in an univariate survival analysis (p=0.018, 27.5 vs 14.6 months) (Figure6)”.

a) Check figure 6 legend and Kaplan-Meier (KM) plots. Legends for panels A/B, C/D or KM plots are switched. Check labels on Y axes of KM plots.

b) Indicate in Fig.6 and 7 legends the meaning of “+” signs. Are they censored cases? If yes why there are no censored cases on Kaplan-Meier plot B (fig.6)?

c) Lines 245-246:”Homogenous positivity for Cyclin A1 was associated with longer OS in the univariate analysis (p=0.044, 65.3 vs 42.2 months). Is it presented on Fig 6, panel D?

Response:

a) Following the reviewer’s suggestion, we checked figure 6 and corrected the mistake. Legends for panels A/B and C/D were switched in the first version of our paper.

b) “+” signs in the Fig. 6 and 7 are censored cases, we corrected the mistake in Kaplan-Meier plot B and added the censored cases.

c) Lines 245-246:”Homogenous positivity for Cyclin A1 was associated with longer OS in the univariate analysis (p=0.044, 65.3 vs 42.2 months). Yes, it is presented on Fig 6, panel D, we corrected statement in the text.

Comment: 2) Lines 234-237: “In that population, the difference in TTP between Cyclin A1 high and Cyclin A1 low patients was even greater (median TTP 26.1 vs 13.0 months) suggesting that
Cyclin A1 expression is predictive of patients responsiveness to the standard first-line chemotherapy regimen (Figure 7F).”

To draw such conclusion authors should additionally compare Cyclin A1 expression in EOCs in relation to tumor platinum-sensitivity and patients response to chemotherapy (RECIST criteria).

**Response:** A comparison of Cyclin A1 staining intensity and percentage of positive cells in dependence of platinum sensitivity has been added to Fig 5 and the lack of significant difference between has been added to the text. Due to the fact that all patients were debulked before adjuvant treatment, and most patients had no macroscopic residual tumor, RECIST criteria are not applicable in most of the cases. An orientating comparison of the patients with PD during adjuvant treatment with all others showed no statistical difference in staining intensity or percentage of positive cells but because of the mentioned obstacles concerning RECIST in this particular case, we feel that this information should not be reported and discussed in the manuscript.

**Comment:** 3) Lines 241-244: “In a multivariate analysis of Cyclin A1 staining intensity, the percentage of Cyclin A1-positive cells, tumor grade, macroscopic residual tumor after debulking, FIGO stage, and age at first diagnosis, only Cyclin A1 high staining intensity was an independent indicator for prolonged TTP (p=0.012). (Supplementary Figure 2).”

Additionally, EOC platinum-sensitivity and peritoneal carcinomatosis should be included in Cox regression analysis. The authors should describe criteria used for inclusion or exclusion of prognostic factors in Cox model. Moreover, the authors should add HR, and lower and higher 95% confidence interval values for all factors included in the final Cox model to Supplementary Table 2.

**Response:** The reviewer asks to add peritoneal carcinomatosis and platinum sensitivity to multivariate analysis. Covariates were chosen based on clinical relevance as described in earlier studies and results of the univariate analyses of our own data. The ratio covariates/events rather than the ratio covariates/patients has direct impact on the reliability of the data (Peduzzi et al 1995). We now added the univariate analysis to the table including peritoneal carcinomatosis and added HR and confidence intervals to both univariate and multivariate analysis. Platinum sensitivity is not a valid covariate because it is defined by TTP and therefore directly dependent of the primary variable. It therefore is not included in multivariate analysis addressing progression and survival (e. g. Pils et al 2013, Zhang, Tang et al 2015, van Kruchten 2015). As mentioned before, the number of 41 and 49 events allows a maximum of 4 covariates in the multivariate analysis. We therefore picked the ones with the lowest p-value in the univariate analysis. The respective supplemental Table 1 and the corresponding legend has been modified accordingly.

**Comment:** 4) Lines 263-264: “Consequently, the data imply that the impact of Cyclin A1 expression on TTP is not dependent of the molecular subtype.”
This conclusion should be supported by the results of the multivariate survival analysis including “C” molecular subtypes of EOC. As there are several different C subtypes, they can be entered into Cox model as dichotomous variable (C1 (most common subtype) vs combined other high-grad subtypes).

**Response:**
Especially for immunotherapy, the actual translation to protein is a pivotal factor. This made us consciously decide not primarily just reanalyse microarray data but to analyze the protein level by IHC. Unfortunately, we do not have corresponding microarray data for the clinical samples analyzed by IHC. This makes it impossible to include the C1 [yes/no]-covariate to our multivariate cox regression analysis. Of course, any conclusions we can draw comparing results from two different data sets analyzed with two different methods have limited informative value. We just wanted to point out that Cyclin A1 is not significantly higher in C1 reducing it to a pure surrogate marker for this molecular subtype. We therefore changed the manuscript in a way, which should make this aspect clearer.

**Discussion**

**Comment:** 1) Lines 325-328: “The longer TTP in patients with higher Cyclin A1 levels might reflect responsiveness to cytostatic treatment rather than an association between more aggressive tumor biology and later-stage and/or metastatic disease and peritoneal carcinomatosis at first diagnosis.”

Update discussion according to the comments to Results.

**Response:**
The respective part of the discussion was modified in accordance to the supplemental Table 1.

**Comment:** 2) Lines 347-349: “Given that C1 is characterized by short TTP, its association with high Cyclin A1 expression implies that the impact of Cyclin A1 expression on TTP is independent of the molecular subtype (Supplementary Figure 3”).

**Comment:** 3) Lines 354-355: “Cyclin A1 acts as predictive marker for response to standard platinum based cytostatic therapy translating into prolonged TTP.”

**Response to 2)/3):**
As mentioned above, the direct inclusion of the Tothill cluster in our analysis was not possible. We modified the respective paragraphs in the results and discussion to clarify the issue.
II: Minor Essential Revisions

Introduction

Lines 115-116: “Currently, we have only sparse data on the impact of Cyclin A1 on proliferation, invasiveness, and resistance to apoptosis in EOC [21].”

Cited work:” Sharma, S.K., at al., Identification of E2F-1/Cyclin A antagonist. Bioorg Med Chem Lett, 2001.11(18): p.2449-52” is cyclin A (cyclin A2) related and it was not performed on EOC.

Response: We thank the reviewer for pointing out this error. We replaced the wrong citation and added.


Material and Methods

Paragraph “Patients and Specimens”

Comment: 1) The authors should indicate if EOC samples (frozen and paraffin-embedded) used for qRT-PCR and IHC were collected from patients before or after chemotherapy.

Response: According to the reviewer’s suggestion, we included the following statement in the Paragraph “Patients and Specimens” “EOC samples were collected before the onset of chemotherapy.

Comment: 2) Lines 142-144:”Samples exceeding the mean expression level plus three standard deviations of the healthy, non-testicular tissue samples were considered positive.” According to figure legends (lines: 388 and 392-393) median +3SD values are marked by horizontal bar in Figure 1 and 2.

Response: We thank the reviewer for pointing out this error. We corrected the mistake in mean+3SD.

Comment: 3) Lines 172-173:”The staining intensities were expressed as weak (1), weak to moderate (1.5), moderate (2), moderate to strong (2.5), or strong (3).” This sentence belong to the “Immunohistochemistry staining” paragraph.
Response: Following the reviewer’s suggestion, we added the sentence to the “Immunohistochemistry staining” paragraph.

Comment: 4) Paragraph “Immunohistochemistry staining”

The authors should describe who evaluated IHC staining and if there were two or more observers how discrepancies were handled.

Response: Following the reviewer’s suggestion, we described in the methods who evaluated IHC staining.

Comment: 5) Paragraph “Statistics”

Provide the name of statistical software used for statistical analysis.

Response: Following the reviewer’s suggestion, we provided the name of the statistical software used for statistical analysis in the Paragraph “Statistics”.

Results

Comment: 1) Lines 213-214: “Homogenous Cyclin A1 positivity observed in 43 of 61 grade 3 specimens but in only one of 11 grade 2 specimens (p=0.005, Figure 4)”.

According to Table 1 and Figure 4 there were 10 grade 2 and 62 grade 3 EOCs (Fig4).

Response: This was obviously a mistake. As reported in Table 1 and depicted in Figure 4 and 5, we had only 10 G2-samples and 62 G3-samples. It was corrected in the text.

Comment: 2) Lines 215-216: “The percentage of positive cells but not staining intensity was significantly higher in the grade 3 specimens (Figure 5B,D).”

Add p value for this comparison in text and add p values for all 4 panels (A-D) in Fig.5.

Response: Following the reviewer’s suggestion, we added p value in text and in all 4 panels (A-D) in Fig.5.
Comment: 3) Paragraph “Cyclin A1 expression is associated with prolonged time to progression”

The authors should calculate and give the mean or median and min-max time of TTP and OS for all patients.

Response:

We added median TTP and OS and the min/max range to the respective paragraph in the results section.

Comment: 4) Lines 228-229: “While grading, age, macroscopic residual tumor after debulking, and peritoneal carcinomatosis/ distant metastasis at first diagnosis had no impact on TTP or OS,…”

The authors should calculate and indicate if FIGO stage and tumor platinum-sensitivity had significant impact on TTP and OS in univariate analysis

Response:

We added FIGO stage in the text and referred to supplemental Table 1, which gives all p-values of the univariate and multivariate analysis. As mentioned above, the platinum sensitivity is highly significant, but should not be calculated because platinum-sensitivity is defined by TTP (direct dependency).

Comment: 5) Lines 238-241: “…online-accessable tool `Kaplan-Meier-Plotter` (www.kmplot.com/ovar[23] to evaluate the impact of Cyclin A1 expression on TTP in an independent data set of 264 patients with serous ovarian cancer after suboptimal debulking and platinum-based chemotherapy. Again, higher Cyclin A1 expression levels were associated with longer TTP (p=0.0088, Supplementary figure 1).”

The authors should indicate the method for cut-off selection and database version used for analysis with KM Plotter as the analysis with following setting (Affy id:205899_at, survival: PFS, split patients by: Auto (best cutoff), restrictions: histology (serous),debulk (suboptimal), and chemotherapy (contains platin), database: 2015 version n=1648) can be performed on 266 patients and yields HR=0.64 (lower risk), p=0.0014 (603 cut-off value). Interestingly, the analysis made on the group of patients after optimal debulking (n=496) gives HR=1.27 (higher risk), p=0.0349 (cut-off value 483), which should be discussed by authors.
Response:

We indicated version and cut-off selection (data base version: 2015 [n=1648], Affymetrix Id: 205899_at, survival: PFS, split patients by median, restrictions: FIGO II, III, IV, histology: serous, debulk: suboptimal, chemotherapy: contains platinum) to text and figure legend. It is important to note that we did not use the ‘auto select best cut-off’-option. This option performs a ROC analysis calculating every single log-rank p-value for each percentile from 25th to 75th to identify the cut-off resulting to the lowest p. If only one data set is used both for the definition of the cut-off and statistical testing using this cut-off, the p-values resulting from this testing are too high. From a statistical point of view, this approach should only be used if the so identified cut-off is validated in a second data set. Using the tool as indicated above, we only see a significant difference in the cohort with suboptimal debulking, but not in the cohort with optimal debulking or in the whole population. This is now mentioned in the text.

Comment: 6) Lines 246-247:”However, in the multivariate analysis, none of the parameters mentioned above was an independent prognostic marker for OS (data not shown”).

There are shown in Supplementary Table 2 “(Supplementary Figure 2)”.

Response: We thank the reviewer for pointing out this error. We corrected the mistake and included the statement in the text (Suppl. Table 1).

Discussion

Comment: 1) Line 307:” (Heiko Schuster, Tübingen, personal communication) [20]” Wrong citation ) “[20]” and the missing reference “Heiko Schuster, Tübingen, , personal communication”.

Response:

We agree that the reference ,Ochsenreither et al.‘ needs not to be quoted here. The reference has been removed. We are not sure whether the personal communication has to be mentioned in the text or in the reference section. We will do it as requested by the editor.

Comment: 2) Line 315: ”…Cyclin A1/CDC2 complex mediates…”

Restle at al. [36] proposed that Cyclin A1/cdk2-mediated phosphorylation of p53 enables stable complex formation with topo I, thereby causing hyper-recombination in p53 mutant cells (Fig.9).
Response:

We agree with this point and have changed the text in the line 315.

Comment: 3) Lines 328-330: “Cyclin A1 directly interacts not only with p53 but also with at least two members of the Retinoblastoma gene product (pRb) pathway, pRb and E2F-1, which regulates proliferation and is itself modulated by p53 [39-41].”

Response:

   a) We thank the reviewer for pointing out this error. We deleted the wrong reference.
   b) We thank the reviewer for pointing out this error. We deleted the wrong reference.

Comment: 4) Line 332: “rRB”

Correct the name.

Response: We thank the reviewer for pointing out this error. We corrected the name in the line 332.

References


Response: We thank the reviewer for pointing out this error. We deleted the reference number 10.


Response: We thank the reviewer for pointing out this error. We inserted the reference again.


**Response:** We thank the reviewer for pointing out this error. We inserted the reference again.

**Comment:** 4) Some references need reformatting according to the journal reference style.

**Response:** According to the reviewer’s suggestion, we reformatted the references according to the journal reference style.

### III. Discretionary Revisions

**Abstract**

**Comment:** 1) Results:”Cyclin A1 was homogeneously expressed in 43 of 61 grade 3 tumor samples and in 1 of 11 grade 2 specimens (p<0.001). Survival analysis showed longer time to progression (TTP) among 49 patients with at least moderate Cyclin A1 expression (univariate:p=0.018, multivariate:p=0.012).”

Was it Cyclin A1 protein expression?

**Response:** We thank the reviewer for this helpful suggestion to improve the clarity of our manuscript. We added the word protein in the text.

**Introduction**

1) **Comment:** 1) Lines 71-72:”Epithelial ovarian cancer (EOC) is the sixth most common cancer and the seventh most common cause of cancer-related death among women worldwide[1],…”

Ovarian cancer is the seventh most common cancer and the eighth most common cause of cancer-related death among women worldwide [http://globocan.iarc.fr/Pages/fact_sheets_population.aspx]; data in Table “Estimated incidence, mortality and 5-year prevalence: women”.

**Response:** We thank the reviewer for this helpful suggestion to strengthen our manuscript. We included this source in the manuscript.
Comment: 2) Lines 73-74:”About two thirds of patients with EOC are diagnosed in an advanced stage with peritoneal or visceral spread [3].”


Response: Following the reviewer’s suggestion, we updated the reference.

Comment: 3) Lines 75-77:”Despite high response rates to first-line systemic treatment, all patients with initially advanced or secondary metastatic disease relapse, develop platinum resistance, and eventually die from the disease[4]”.

Approximately 80% of women with advanced ovarian cancer will have tumour progression, or more commonly a recurrence, which is usually eventually fatal due to the emergence of drug resistance [Luvero D, Milani A, Ledermann JA. Treatment options in recurrent ovarian cancer: latest evidence and clinical potential. Ther Adv Med Oncol. 2014 Sep;6(5):229-39.doi:10.1177/1758834014544121.].

Response:

We inserted the requested reference.

Results


Response:

Link was corrected in the text.

Comment:2) Table 1, Last row ”Primary platinum sensibility”. Change “sensibility” to “sensitivity*”

Response:

The term “sensitivity*” can now be found in Table 1 of our revised manuscript.

Comment: 3) Legend for Figure 1

Add the median+3SD value in the legend to this Fig.
Response: Following the reviewer’s suggestion, we added mean+3SD value in the legend to Figure 1.

Comment: 4) Figure 2:

1) Several names of healthy tissues on X axis are cut.

2) Add label to Y axis e.g. CCNA1 expression (%).

3) Add the median+3SD value in the legend to this Fig.

Response: We thank the reviewer for pointing out this error.

Figure 1 and 2 were redone with new lettering and new threshold lines.

Comment: 5) Supplementary Figure 3

1) Figure Legend, Describe the meaning of red horizontal lines on dot plots. Are they median or mean values?

2) Add Label to Y axis e.g. CCNA1 expression.

Response: Following the reviewer’s suggestion, we described the meaning of horizontal lines on dot plots (which are medians, because the statistical testing was conducted with a non-parametric test) and added a label to the Y axis (which is identical to Fig 1).

Responses to Reviewer #2 (green color)

The authors present evidence to support the use of Cyclin A1 as a member and potential target for T cell therapy. They present evidence that cyclin A1 is associated with longer survival. The manuscript needs some upgrades to be considered for publication. It appears the authors used online tools but have limited statistical support or experience with expression data analysis.

Major Compulsory Revisions

Comment 1) It is not clear which datasets were used for the analysis. Which datasets were used to analyze probe set 205899-at for Figure 1? There are many other tools to evaluate cyclin A1 in
ovarian cancer and many datasets. One relatively easy one to use is OVMark. This may or may be not useful at the RNA level compared to the protein level, but should be considered and presented in the manuscript. The authors should clarify in the datasets used to evaluate Cyclin A1 and evaluate cyclin A1 in as many datasets as possible given the large amount of data publically available.

Response:

1) We added the GSE of the panel with different tumor entities to the material and methods section. We added the information, which panels were used for Figure 1 to the respective paragraph in the results section.

We were testing both OVMark by Stephen Madden and the Kaplan-Meier plotting tool by B. Györffy to confirm our observation with a second independent data set. We figured out that there was a substantial overlap in data sets analyzed by the two online tools. One problem we faced using the OVMark tool was that the annotation of the samples seemed to be insufficient in a way that when we just checked the box ‘serous’, of 996 samples with CCNA1 represented by the respective microarray platform, one third including the TCGA data set was excluded from the analysis. When additionally ‘chemotherapy’ was checked, this left us with 42 cases. The selection ‘serous’ AND ‘no neoadjuvant chemotherapy’ AND ‘Platinum-containing chemotherapy’ left us with the two data sets from Tothill et al and Ferris et al., together 215 cases. The calculator by Györffy contains many of the datasets, which are also used by Madden et al. including the data from Tothill, Denkert and the TCGA. However, the stringent case selection as described above still left us with enough cases to come to statistically relevant results. Furthermore, only one platform is used (the Affy U133A/U133Plus2) with only one probe set representing CCNA1, which already has been validated by qRT PCR in the past. These two reasons together with the issue of the overlapping use of many data sets made us use only the tool by Györffy. Even though the mentioned two data sets by Ferris and Tothill analyzed by OVMark actually detected a significant benefit for high CCNA1 expression in PFS (p=0.02), because of the reasons discussed, we decided not to report this observation. However, we added information about the data sets represented by the Kaplan-Meier-Plotter to the results section.

Comment 2) The y-axis for Figure 2 needs to be clarified. Are the data normalized to Human testis relative to GAPDH?

Response: Expressions are first calculated as copies per copies of housekeeping gene GAPDH. The figure gives expressions relative to expression in testis (=100%). We indicated this in the figure legend.
Comment 3) Was residual disease not an independent predictor of OS in the patient cohort used for IHC? It seems based on lines 228-231 that it is not. Why? This differs from most ovarian cancer cohorts and if so, could explain why Cyclin A1 appears to be independent of residual disease in the multivariate analysis. The numbers from the univariate analysis for each parameter, stage, residual disease, etc. should be added to the Supplemental figure to be able to evaluate the cohort. In the discussion, lines 324-325 say that Cyclin A1 predictions were stronger than residual disease, but residual disease has not signal in this cohort, so this whole argument is weak as this dataset may have biases not seen in most ovarian cancer datasets.

Response: 3) We added the results of the univariate survival analysis to the supplemental table 1 as requested by reviewer 1 and 2.

The absence of other statistically significant predictors (residual tumor, FIGO III/IV, peritoneal carcinomatosis) for TTP and OS in our cohort has been discussed extensively in our group. To see whether our clinical data was implausible or the cohort would not represent a typical patient population we compared our clinical patient characteristics with the characteristics of the TCGA data set of 488 patients. These two populations were almost identical [this study vs TCGA], mean age 59 vs 60, FIGO stage II/III/IV [%] 4/82/14 vs 5/79/16, grade 2/3 [%] 14/86 vs 12/88, residual tumor yes/no [%] 72/28 vs 72/28, platinum sensitivity yes/no [%] 67/33 vs 69/31). Interestingly, except the age of the patients, none of the mentioned patient characteristics except platinum sensitivity had statistical impact on OS neither in the univariate nor in the multivariate analysis. We interpreted this observation as a confirmation for us that the absence of clinical predictors for TTP and/or OS not necessarily implies that the patient cohort we used was not representative.

Residual disease is now shown in the supplemental Table 1, and the respective paragraph in the discussion has been modified.

Comment 4) Why is high Cyclin A1 expression associated with longer survival in the presented data, but has high expression in the C1 category, which has short survival? Is this due to the modest to borderline statistical significance of the Cyclin A1 association with survival?
Response: It is important to state that high Cyclin A1 expression is not associated with longer survival but with longer TTP. Aim of the study was the analysis of Cyclin A1 on protein level. As the reviewer mentions himself, there are discrepancies comparing expression on mRNA and protein level, and we have to take into account that the results of both methods, IHC and microarray expression analysis, reflect the actual expression levels in an ordinal or at least non-linear way even though in our hands the two methods provided results, which were correlated non-parametrically. Therefore, we decided not primarily just reanalyse microarray data but to analyze the protein level by IHC. Accordingly, any conclusions we can draw are these comparing results from two different data sets analyzed with two different methods. We just wanted to point out that Cyclin A1 is not significantly higher in C1 reducing it to a pure surrogate marker for this molecular subtype. We modified the respective part of the results section and, more important, changed parts of the discussion explaining why we could not include the molecular subtypes into our multivariate analysis discussing the limitations of this comparison.

Comment 5) Why do the authors think that Cyclin A1 is a good target for immunotherapy if is associated with better responses? Would these responses be improved further by targeting Cyclin A1? Or is the expression pattern what is really important for T cell therapy? What is the importance between the prognostic impact of Cyclin A1 and its potential use for immunotherapy?

Response:

5) This two aspects (1) high, often homogenous expression of a protein, which is normally selectively expressed in testis, makes Cyclin A1 a good T-cell target and (2) the differences in response duration after adjuvant or palliative chemotherapy, are two independent aspects. We do not want conclude that because of the longer TTP of Cyclin A1 expression in tumors, this target is a good one. We modified the manuscript in a way that these two aspects are clearly separated in the discussion.

Minor Essential Revisions:

Comment 1) The manuscript has a number of grammatical errors and couple of typos.
Response: We thank the reviewer for this notice. We corrected the grammatical errors and typos.

Comment 2) Figure 6A and 6B appear mixed up relative to the Figure legend.

Response: We thank the reviewer for this observation. We corrected the order of the figures.

Comment 3) The key for figure 6 is too small and hard to read. Actually, the font size in Figure 6 should be generally increased.

Response: We thank the reviewer for this observation. We increased the font size in all figures.

Responses to Reviewer #3 (pink color)

Major Compulsory Revisions

Comment 1) Abstract is difficult to follow—T cell; time to progression, etc.

Response: We tried to modify the abstract to give more explanation of the different aspects of the work. However, this led to an exceeding word count. We hope, it is clear enough with the few small modification we made.

Comment 2) Spelling errors throughout

Response: We thank the reviewer for attentive reading of the manuscript. We double-checked the whole manuscript for the spelling errors.

Comment 3) Expression levels were presented as copies per copies of GAPDH. (see Page 5, Line 154)- this phrase is unclear.

Response: We specified the sentence.
Comment 4) Page 4, Line 107 - a citation is needed here.

Response: We added the citation page 4, line 107.

Comment 5) Figure 2, x-axis labels are not clear.

Response: We thank the reviewer for this suggestion. We wrote the labels in a way to be clear.

Comment 6) Interpretation of several of the figures is unclear.

Response: We thank the reviewer for this remark. We rewrote the description of several figures.

Comment 7) Figure 3: should not representative images of various stages/grades of ovarian cancer be shown in addition along with normal ovarian sample?

Response: There is no expression in normal ovarii, so we do not think that would be informative to show normal ovarian sample.

Comment 8) Grad # should be Grade in figures.

Response: We thank the reviewer for pointing out this error. We corrected the error.

Comment 9) HGOC versus HGEOC: is there a difference between these two nomenclatures? Perhaps consistency in labelling may be helpful.

Response: Following the reviewer’s suggestion, we wrote consistently HGOC throughout the whole manuscript.