Response to Reviewer 1 Comments

The Review is not always clear and comprehensive. It would greatly benefit of some IHC examples. The description of the biomarkers is a "list" without internal connections and clear role in platinum resistance. References are poor and often not up-to-date. The authors should pay attention to the abbreviations and the nomenclature that should be consistent throughout the text.

We have corrected the English, remove unclear sentences, added and reorganise the manuscript to show relevance of biomarker to platinum resistance or as prognostic marker. We have corrected any typos or errors in abbreviations and nomenclature.

Specific comment

Point 1: I would suggest a brief description of the two main subtypes of epithelial ovarian cancers.
Response 1: High-grade serous ovarian cancer (HGSOC) is the most common and aggressive form of EOC, which account for about ~70% of all cases and it is the leading cause of cancer-related death among all gynecological cancers worldwide. Less common types of epithelial ovarian cancers include: endometrioid carcinoma which consists about ~20% of EOC and occurs more commonly in women with endometriosis; low-grade serous ovarian carcinoma (LGSOC) which is a slow growing tumour that accounts for about 5% of EOC; mucinous carcinoma, which are more distinct and tend to be large (around 8 inches or 20 centimeters); and ovarian clear cell carcinoma (OCCC) accounts for approximately 5% of all ovarian carcinomas and is characterized by high recurrence rate.

Point 2: It would be helpful for the readers to include some examples of histological examinations.
Response 2: Added Figure 1 into the manuscript which shows the different histological features of the common subtypes of EOC.
Point 3: Table 1: It reads very busy. Perhaps, the authors can prepare two tables, one for each subtype (EOC vs HGSOC). This would allow to eliminate a little bit of text and simplify the Table.
Response 3: Separated Table 1 into one table for EOC and another for subtypes of EOC- OCCC and HGSOC.

Table 1. Summary of IHC biomarkers associated with chemoresistance and prognosis in EOC

Biomarker	FIGO Stage	Study Design	Cut-off Score of expression	Outcome Measured	Clinical Indicator	Reference
VEGF	I-III	NR	10%	PFS, OS	Early detection of poor prognosis	[26]
P53	I-IV	NR	≥ 10%	OS	Poor survival	[29]
MIB1/KI67	1-IV	NR	≥ 50%	PFS	Improved PFS	[30]

Chk2	I-IV	RC	≥ 50%	Chemotherapy response	Predicts good response to platinum-based chemotherapy	[32]
Notch3	I-II	RC	≥ 50%	Chemotherapy response, OS	Chemoresistance, poor OS	[36, 37]
COX-1, COX- 2	NR	RC	> 30%	OS	Poor OS indicator	[48, 49]
BRCA1	NR	RC	≥10%	PFS, OS	Negative prognostic factor	[50]
PD-L1 & PD-L2	NR	RC	>20%	PFS, OS	Negative prognostic factor	[51]

Abbreviations: EOC, Epithelial Ovarian Cancer; FIGO, International Federation of Gynecology and Obstetrics; IHC, Immunohistochemistry; RC, retrospective cohort; OS, overall survival; PFS, progression-free survival; DSS, Disease-specific survival; NR, not reported.

Table 2. Summary of IHC biomarkers associated with chemoresistance and prognosis in HGSOC and OCCC

Biomarker	Subtype	FIGO Stage	Study Design	Cut-off Score of expression	Outcome Measured	Clinical Indicator	Reference
CD133	HGSOC	I-IV	RC	≥ 10%	OS	Early detection of poor survival	[27, 28]
MAD2	HGSOC	I-III	RC	≥ 50%	PFS, OS	Risk of mortality and recurrence	[31]
IGF1R	HGSOC	I-IV	RC	≥ 5%	PFS, OS	Improves PFS and OS	[33]
PGD2	HGSOC	NR	RC	≥ 50%	PFS, response to chemotherapy	Predict good prognosis, PFS and chemotherapy sensitivity	[34]
ERCC1	HGSOC	NR	RC	> 50%	PFS, OS	Predict longer PFS and OS	[35]
Aldh1a1	HGSOC	NR	RC	> 20%	OS	Poor prognostic marker	[40, 41]
Hnf-1β	HGSOC	NR	RC	> 30%	PFS	Chemotherapy resistance indicator	[46, 47]
FOXP3	HGSOC	I-IV	RC	≥ 50%	DSS	Positive prognostic factors	[52]
TNFR2	HGSOC	NR	RC	>50%	PFS	Shorter PFS	[104, 105, 53]
STAT3	HGSOC	NR	RC	> 50%	PFS	Shorter PFS	[54]
GPC3	OCCC	I-II	RC	> 15%	OS	Poor prognostic marker	[38, 39]
Aldh1a1	OCCC	NR	RC	> 20%	OS	Poor prognostic marker	[40, 41]

Hoxa10	OCCC	I-III	RC	≥ 50%	5-year survival	Poor indication of 5-year survival	[42, 43]
Arid1a	OCCC	NR	NR	0	PFS, OS	Poor prognostic marker	[44, 45]
Hnf-1β	OCCC	NR	RC	> 30%	PFS	Chemotherapy resistance indicator	[46, 47]

Abbreviations: HGSOC, High-Grade Serous Ovarian Cancer; OCCC, Ovarian Clear Cell Carcinoma; FIGO, International Federation of Gynecology and Obstetrics; IHC, Immunohistochemistry; RC, retrospective cohort; OS, overall survival; PFS, progression-free survival; DSS, Disease-specific survival; NR, not reported.

Point 4: lines 100-104: this part in unclear. Please, modify it.
(In addition, various molecular genetic alterations and genome wide mutations have been associated with ovarian cancers of different types and grade of malignancy, which appear to be associated directly with disease prognosis and loss of chemosensitivity. Chemotherapy resistance is linked to subsequent process, which involve alterations in the transport and cellular turnover of the medicine, DNA repair systems and also effect other nonspecific cytoplasmic defense systems [21]).
Response 4: In addition, various molecular markers including biomarkers derived from genomic abnormalities (such as gene mutations, copy number abberations and DNA methylation) have been associated with different types of EOC. Some of these biomarkers can predict disease prognosis and chemosensitivity. Chemotherapy resistance is linked to multiple mechanisms which includes changes in the transport and cellular turnover of the drug as well as alterations in cytoplasmic defense systems and DNA repair mechanisms [21] leading to loss of treatment sensitivity.

Point 5: lines 127-128: this part in unclear. Please, modify it.
(Which make the examination of the cancer microenvironment is possible and easily to be studied clinically)
Response 5: Hence, studying the biomarkers within the tumour tissue microenvironment may yield better success for potential discovery of relevant and critical biomarkers for prognostication and as therapeutic target.

Point 6: lines 172-173: this statement is not correct. A protein is not a tumor suppressor gene. Please, modify it.
(The 53 kDa nuclear protein produced by this tumour suppressor gene, which is located on the short arm of chromosome 17, regulates cell growth)
Response 6: It is a tumour suppressor gene located on the short arm of chromosome 17, which regulates cell growth.

Point 7: line 201: MIB1 is an antibody used in IHC and as a consequence cannot be overexpressed. This error is present several times and generates confusion. Please, modify it throughout the text.
(Numerous studies have shown a link between MIB-1 (Ki-67) overexpression and a growing number of malignancies, including ovarian, colorectal, gastric, and breast cancers)

Response 7: The whole content on Ki-67 and MIB-1 has been rewritten to ensure no confusion in any statement on it being overexpressed.

Ki-67 gene (10q25) is located on the long arm of human chromosome 10 [73]. The quantification of the reactive expression of Ki-67 antigen using immunohistochemical tool has been demonstrated to provide an estimate of the tumor's proliferative capacity and therefore been widely utilized as a reliable prognostic marker in almost all types of cancers including those of the lymphatic system, lung, brain, breast, cervix, uterus, ovary, and soft tissue sarcoma. The key feature of this biomarker is its expression is absent in quiescent state of cells (G0) and expressed in all of the active cell cycle phases (G1, S, G2, and mitosis) in proliferating tissues. The strongest areas of Ki-67 immunostaining are assessed, and all discernible nuclear staining—regardless of intensity—is considered as positive immunoreactivity. Normally, immunostaining is restricted to the nucleus, and only mitosis is associated with cytoplasmic positivity. Using a high-power microscope objective, the percentage of positively stained cells (x400) expressed is defined as Ki-67 labelling index (Ki-67 LI). Numerous malignancies, including ovarian cancer, have been demonstrated to have a poor prognosis with high Ki-67 LI [73, 74].

Mindbomb E3 ubiquitin protein ligase1 (MIB-1 antibody) Immunohistochemistry (IHC) is a relatively new technique for determining the Proliferative Index (PI) of a neoplastic lesion [75, 76]. The monoclonal mouse antibody MIB-1 is the standard for demonstrating PI in formalin-fixed, paraffin-embedded specimens for the Ki-67 antigen. It reacts with the nuclear protein Ki-67 antigen, which comes in two isoforms with molecular masses of 345 and 395 kDa. Numerous studies have linked the presence of this IHC marker in EOC with other prognostic indicators such histologic subtype, tumour grade, FIGO stage, treatment response, as well as with survival rates [75, 76]. In addition, the mean MIB-1 index in HGSOC was found to be 55.4% compared with 23.0% in low-grade ovarian serous cancer [78]. Similarly, another study on the MIB-1 proliferative index (an independent predictor of lymph node metastasis) have shown a significantly lower value in low-grade ovarian cancer (16.3%) when compared with HGSOC (47.8%) [79].

Elevated Ki-67 LI was linked to high grade tumours (69.9%), high grade serous tumours (65.34%), and advanced FIGO staging (70.6%) in a study involving 202 women [75, 77]. However, Ki-67 LI and CA 125 levels did not significantly correlate. The growth fraction of a tumour cell population can be determined with outstanding cost-effectiveness using the marker Ki-67. When paired with Ki-67 LI in the histopathology report, histological grade and FIGO stage of EOC can aid in the diagnosis of subtype distinction, prognostication, determining whether adjuvant treatment is necessary, and survival analysis [75]. In a study involved seventy-three patients with EOC, Ki-67 expression in combination with survivin and Topoisomerase IIα was evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections in relation with response to chemotherapy. Nuclear staining for all antibodies was scored in a 3-layer system and staining >10% was accepted as expression. It was found that Ki-67 was related to poor OS ($p=0.005$), however, there was no association between Ki-67 expression and histological subtype, stage or grade of ovarian cancer [80].

It has been well established that Ki-67 immunostaining of human tumours has diagnostic and prognostic significance. The Ki-67 immunostaining has been carried out on a variety of histological and cytological specimens, including frozen sections, smears, cell suspensions, etc. However, the limitation of this epitope is that it does not survive conventional histopathological fixation, such as in formaldehyde or alcohol [75].

Regular inclusion of the Ki-67 LI / MIB-1 IHC marker as a diagnostic and prognostic feature in histopathology reports would pave the way for a better understanding of biological behaviour and the modification of treatment plans. The Ki-67 antigen/ MIB-1 antibody reactive staining therefore can be employed as a diagnostic and predictive tool to direct the clinical care of ovarian cancer. Recent advances in methodological refinement have made Ki-67 antigen immunostaining a promising goal for PI.

Point 8: lines 204-205: this part in unclear. Please, modify it.
(Higher FIGO stage (P=0.003) and histological grade (P=0.0001) increased the frequency of MIB-1 (Ki-67) positive expression, and adenocarcinoma showed a substantially different distribution of MIB-1 (Ki-67) positive and negative tumours).
Response 8: This sentence has been removed and replaced with above paragraphs as in Point 7.
Elevated Ki-67 LI was linked to high grade tumours (69.9%), high grade serous tumours (65.34%), and advanced FIGO staging (70.6%) in a study involving 202 women [75, 77].

Point 9: line 211: what does it mean 16.3 vs 47.8%?
(Similarly, another study identified a lower MIB-1 proliferative index in invasive low-grade ovarian cancer compared with HGSOC (mean, 16.3 vs 47.8%) [79].)
Response 9: Similarly, another study on the MIB-1 proliferative index (an independent predictor of lymph node metastasis) have shown a significantly lower value in low-grade ovarian cancer (16.3%) when compared with HGSOC (47.8%) [79].

Point 10: lines 297-302: this part in unclear. Please, modify it.
(GPC3 expression was only infrequently seen in the other histological subtypes of ovarian adenocarcinoma, such as mucinous (4%) endometrioid (5%) and HGSOC, in a study of 213 cases of ovarian adenocarcinoma (11%). Although GPC3 expression was significantly associated with poor OS in cases of stage III/IV clear cell adenocarcinoma, there was a negative correlation between GPC3 expression and clinicopathological aspects of clear cell adenocarcinoma, such as tumour stage, lymph node metastasis, peritoneal dissemination, and death rate.)

Response 10: In a study of 213 cases with different subtypes of EOC, the GPC3 positive expression was demonstrated mainly (44%) in ovarian clear cell carcinoma (OCCC), and less in other subtypes including mucinous (4%), endometrioid (5%) and HGSOC (11%). Although GPC3 expression was significantly associated with poor overall survival in advanced (stage III/IV) OCCC, there was a negative correlation between GPC3 expression and clinicopathological aspects, such as tumour stage, lymph node spread peritoneal metastasis, and death rate [39].

Point 11: lines 310-311: this part in unclear. Please, modify it.
(Many studies have associated this biomarker with tumour initiating potential of cells and poor prognosis of the disease [94, 95].)

Response 11: The entire paragraphs for *Aldehyde Dehydrogenase (ALDH1) have been rewritten.*

Is a catalase that oxidizes aldehyde-containing molecules and ALDH, therefore it plays an important role in cellular homeostasis. In cancer cells, it assists in both energy production via retinoic acid (RA) synthesis, and in deactivation of drug molecules (by action on the aldehyde group) [40]. A growing body of research indicates that ALDH may not only be utilised as a marker for stem cells but may also control cellular processes like self-renewal, growth, differentiation, and radiation and drug resistance. According to recent research, both healthy and cancer cells with high levels of ALDH1 activity have the capacity to serve as stem cells, as well as the capacity for self-renewal and stress resistance [94, 95]. Many studies have shown that high expression of ALDH1 is found to be associated with increased capacity for sphere formation, tumorigenicity, and invasiveness [94, 95].

There is strong evidence to support the idea that the specific isoform(s) of ALDH expressed in tissue determines its function as a stem cell marker. The cytosolic enzyme needed for RA production belong to the ALDH1 family, and ALDH1A1 have received a lot of attention. Both normal stem cells and stem-like cells that initiate tumours have their biological functions controlled by ALDH1A1, which encourages tumour growth and chemotherapy resistance [94, 95].

Point 12: lines 316-318: this part in unclear. Please, modify it.
(There was no significant link between the expression level of ALDH1A1 and age or with any FIGO clinical stage, and the medians of ALDH1A1-positive rates in serous and clear cell adenocarcinoma patients were determined to be 20% and 15%, respectively.)

Response 12: This statement has been removed and rewritten.

A study by Kuroda et al. firstly reported that ovarian cancer can also be prognosticated poorly based on high ALDH1A1 expression in the immunohistochemistry of the ovarian tissue. Higher ALDH1 expression levels in ovary cancer cases were found to be associated with a worse prognosis in both serous ($p=0.006$) and clear cell adenocarcinoma ($p=0.047$) cases, according to immunohistochemical staining of a total of 123 EOC tissues [41]. ALDH1, hence is a marker for ovarian cancer stem and that the degree of ALDH1 expression may be a potential diagnostic for predicting a bad prognosis.

Point 13: lines 354-357: this part in unclear. Please, modify it.
(reported that HNF-1β was significantly found over-expressed in OCCC and longer PFS ($p=0.01$) and OS ($p=0.02$) have been linked to HNF-1 depletion (lack of nuclear staining in IHC). Therefore, HNF-

1β loss (absence of nuclear staining in IHC) has been attributed to good prognosis for women with OCCC [47].)

Response 13:

The whole paragraphs for HNF-1β have been rewritten.

HNF-1β is a homeodomain-containing transcription factor which binds to the same DNA sequence as homodimers or heterodimers [97]. Numerous genes are known to be regulated by it, either directly or indirectly [98]. Majority of ovarian cancer clear cell carcinoma (OCCC) over-express HNF-1β, therefore HNF1β over-expression is likely to be helpful for the diagnosis of OCCC [46]. The presence of HNF1-binding sites at numerous OCCC-specific hypomethylated genes further supports this notion. In addition, a motif analysis found that HNF1 binding motifs are significantly enriched in genes that comprise the OCCC signature [46]. In two separate large cohort studies, one on Australian population known as Australian ovarian cancer women (AOCS) and in another study on Japanese population known as high-volume Japanese university clinical network (JIKEI), the over-expression of HNF-1β was found to be in OCCC subtypes and have been associated with significantly longer PFS (p=0.01) and OS (p=0.02) [47].

Mandai et al. demonstrated that HNF-1β increases OCCC cell survival by enhancing Reactive Oxygen Species (ROS) resistance. Hence, HNF1β inhibition with some type of inhibitor, such as the microRNA mir-802, may yield therapeutic effect by annulling ROS resistance. The HNF1β-induced cell survival according to the author was shown to be glucose-dependent. Therefore, glucose metabolism may be a therapeutic target in OCCC with high HNF-1β expression.

Point 14: lines 396-399: this part in unclear. Please, modify it.

(The 5-year survival rate in the other hand, in women with high expression of PD-L1 was significantly worse than those with low expression. This suggest that the aberrant expression of PD-Ls on tumour cells impairs antitumor immunity, resulting in the immune evasion of the tumour cells.)

Response 14: Women with high PD-L1 expression have shown poor 5-year survival rate than those with low PD-L1 expression. This suggest that the higher expression of PD-L1 on tumour cells leads to impaired antitumor immunity [105].