Response to the Reviewers
ESPS manuscript NO: 18596
Title: Alpha1b adrenoceptor expression is a marker of reduced survival and increased tumor recurrence in patients with endometrioid ovarian cancer

We thank the reviewers for their constructive comments that we believe has improved the paper. Our response to each reviewer is shown below.

REVIEWER 1
Reviewer's code: 00742121
Reviewer's country: Greece

Comments to Authors
This is a well written paper worthy of publication after minor revision, according to the comments listed below. - Abstract:The "Objective" section should be shortened. -The term "cancer specific survival" used throughout the text should be probably replaced by "ovarian cancer specific survival". - There are some other terms used, that should be better changed (see comments in the manuscript). - The authors should discuss possible limitations of alpha adrenoreceptor antagonists in clinical practice, due to adverse effects of these agents.

Author Response:
The term "cancer specific survival" has been changed to ''ovarian cancer specific survival''.
The implication of treating patients with alpha adrenoreceptor antagonists has been addressed in the Discussion; candidate drugs and side effects is provided.

REVIEWER 2
Reviewer's code: 00742373
Reviewer's country: United States

The manuscript titled "Alpha1b adrenoceptor expression is a marker of reduced survival and increased tumor recurrence in patients with endometrioid ovarian cancer" studied alpha-1B, alpha-2C, and beta-2 adrenoceptor in ovary cancer patients and analyzed their relationship with the patients mortality and recurrent of the cancer. Results have shown an association between expression of alpha-1B and patients survival as well as tumor recurrence in some specific type of ovary cancer. The authors concluded that alpha-1B adrenoceptor protein predicts the outcome of endometrioid type ovarian cancer. This manuscript choice a very important disease-ovary cancer and studied in the molecular biology level-adrenergic receptor expression. Patient number is appropriate, experimental methods are suitable. English is well written. Structure of the manuscript is good. Figures and tables are clear. Major concern: Normal expression or standard expression of adrenergic receptor is not included in the study nor related referenced discussed in the manuscript. We say high (up) expression or low (down) expression, usually is

compare with the normal group or control group. Reviewer noticed the authors only compared between the ovary cancer groups. We know that tumor type is usually associated with the outcomes and recurrence of the tumor. The author analyzed the expression of adrenergic receptor as present and absent and resulted in the p value in an overall of ovary cancer group. We don't know if the individual tumor type expression of adrenergic receptor is normal expression or abnormal expression. So, reviewer suggest add another group of adrenergic receptor expression from the ovary tissue without cancer. I believe the tissue may be obtained from previous patients with other disease. By this way, hopefully we could find out it is real up or down expression of the adrenergic receptors. As well in the mortality analysis, the study of a different group other than ovary cancer will demonstrate whether different expression of Alpha-1b is related with mortality just in ovary cancer patients or in patients other than ovary cancer. Minor concerns: ? Some English spelling and grammar corrections. ? It is hard to understand figure 2 and figure 3. Suggest explain in more detail.

Author Response:

Adrenergic receptor expression in ovarian tissue: We appreciate the reviewers point about using the term over and under-expression without comparison to a normal ovary tissue control. For clarity, the staining intensity of each biomarker will be substituted for the terms 'over- and under-expression' as this removes the inference of what might be expected relative to normal ovary tissue.

Turning to the matter of whether alpha and beta AR expression occurs in ovary tissue the answer appears to be affirmative. Most characterisation and localisation studies of adrenergic receptor isoforms has been in rodents. MT Itoh and B Ishizuka (Mol Cell Endocrinol 2005;240(1-2):58-63) verified the localisation of alpha1b AR protein in rat ovarian tissue using immunohistochemistry and western blotting: a band with a molecular weight of 80kDa was detected. In addition hamster ovarian tissue has been used as a positive control tissue in other studies including that by AP Ford et al (Br J Pharmacol 1997;121)6):1127-35). KJ Fohr et al report he presence of alpha adrenergic receptors in humans (J Clin Endocrine & Metab 2013; 76(2). Beta2 AR has been localised in human ovarian tissue using multiple lab techniques: C Merz et al (J Ovarian Res 2015 8: 8 doi:10.1186/s13048-015-0136-4).

It is suggested that adrenergic receptor immunohistochemistry is performed on a cohort of normal ovary samples. We feel that this is unnecessary for a couple of reasons. Firstly, we accept that the tumour type is usually associated with prognosis and if our findings were due to tumour type alone we would expect to see similar tumour recurrence outcome data for all patients classified with the same tumour type regardless of biomarker expression. But we observed different levels of staining intensity between patients within the epithelioid group: those showing moderate to strong staining intensity showed an increased risk of cancer recurrence. Secondly, we have amended the terminology used when referring to levels of adrenergic receptor protein expression. Previous studies suggest that alpha1b expression is present in ovary tissue and that the receptor is involved in cell proliferation; our findings are consistent with this.

To answer the reviewers question asking if other cancer tissues have been investigated for adrenergic receptor expression, the answer is yes. We previously published a study of breast cancer where we found increased alpha1b staining in tumour cells is associated with poor survival and increased tumour recurrence (DG Powe et al. Breast Cancer Res Treat 2011; 130:457-463).

Figure 2 shows the likelihood of a) ovarian cancer specific survival and b) tumour recurrence as a function of time after diagnosis for the whole patient cohort (all tumour types). We used the Kaplan-Meier technique (in the statistics program SPSS) to model and compare how patients fared if they have alpha1b staining (green curve) or not (blue). The plot shows alpha1b positive patients have reduced survival probability compared to their alpha1b negative counterparts. As an example, if a vertical line is drawn at 100 months survival time (Fig 2a) alpha1b patients have a 25% probability compared to a 45% survival probability in alpha1b negative patients. Separate sub-analysis of different tumour types revealed this finding is strongest in the epithelioid patient group.

Fig 3 is similar but tests the association between different patterns of ovarian cancer specific survival and tumour recurrence in subgroups of patients that have been defined according to alpha1b and alpha2c adrenergic receptor expression patterns. This is based on the proposal that alpha1b drives cell proliferation (mitogenic) in activated cells whereas alpha2c is inhibitory. The graph shows that patients with alpha1b staining (regardless of alpha2c expression; groups 1 &2) have a reduced survival probability and are more likely to develop tumour recurrence.

The figure legends have been amended to assist clarity.

English spelling and grammar has been used rather than US.

REVIEWER 3

Reviewer's code: 00742253

Reviewer's country: Lithuania

I would like to congratulate the Authors with this great work. I am sure the readers will accept this paper with a big interest. Would you please explain this sentence for me, thank you. "Alpha1B adrenoceptor protein predicts increased risk of tumor recurrence and reduced mortality in patients with endometrioid type ovarian cancer."

Author Response:

This sentence is explained by the graphs shown in Figures 2 & 3 and the Discussion. A more detailed explanation of these graphs is provided for Reviewer 2 (above). In summary, patients with ovarian tumours that stain positive for the alpha1b receptor protein tend to die sooner of their disease compared to patients that have tumours not expressing this protein. In addition and perhaps because of this, patients with alpha1b positive cancers also show earlier development of tumour recurrence. This observation was most significant in patients with the epithelioid tumour type.