Ms. Treena Guo
Assistant Editor, Cancers

Barcelona, June 6th 2019

Dear Ms. Guo,

Please find attached our revised review article intended for the upcoming special issue on "Uveal Melanoma" entitled “Uveal melanoma, angiogenesis and immunotherapy, is there any hope?”.

We want to extend our sincere gratitude to you and the three reviewers for considering our manuscript, and for suggesting changes that greatly have improved the clarity of the text, its comprehension and the scientific content.

Below, we provide the detailed list of text changes and answers required that we hope to conduct at positive final decision of this manuscript. We have made corrections to the main text to meet the requirements and suggestions proposed by each referee. All changes to the main text have been made using the “Track Changes” option in Microsoft Word. The specific responses to the comments raised by the Editor as well as Reviewer 1-3 follow below.

Editor
- “Please list the first ten authors then use the «et al.», thank you”

We have listed the first ten authors and the used et al.

Reviewer 1
- “In page 9, line 293, “in dis matter” should be corrected by “in this matter”

Thank you for pointing this mistake. We have corrected it accordingly.

- “A section (or a couple of sections) describing angiogenesis in general and then immunotherapy in general, should be included. The authors have described all the processes in relation to UM or Melanoma, however, a more general introduction to those approaches (with the latest strategies) including references for other tumors is desirable.”

Thank you for this suggestion, as we acknowledge the text was unclear. The first section of “Angiogenesis in melanoma” is referred to angiogenesis in general, and it is not until paragraph 3 of this section where we focus more specifically in angiogenesis in melanoma. Much of the preclinical work we show was not done in melanoma but in other tumours. In order to clarify this, we have specified the type of tumours in which each work was carried out.

- “The authors should comment previous results in other diseases (not just melanoma) at the preclinical and clinical level to support this hypothesis”.


We have added the following section to comment on the clinical findings in other diseases:

“The strategy of combining immune-checkpoint inhibitors with antiangiogenic drugs has been studied in other tumours [192]. For instance, three recent phase III studies combining anti-PD1/PDL1 with antiangiogenic therapies showed promising results in advanced renal cell carcinoma [193,194]. Pembrolizumab and axitinib increased overall survival compared to standard first-line sunitinib [194], and the combination of avelumab-axitinib increased progression-free survival [193]. Atezolizumab-bevacizumab also showed an increase in progression-free survival, although the survival data were still immature. In non-small cell lung cancer, the combination of chemotherapy with bevacizumab and atezolizumab increased progression-free survival and overall survival compared to chemotherapy and bevacizumab alone [195,196]. “ We have also specified in what tumour types the preclinical findings were carried out, to show that some of the findings are not only specific to melanomas.

We are also thankful for the suggested reports, as they will enrich the content of the review. We have included the first two.

- “The section focused on angiogenesis is significantly larger than the section for immunotherapy, it would be better to balance a little both sections.”

It was our intention to focus more on angiogenesis than on immunotherapy, as the number of reports and reviews of immunotherapy in melanoma was significantly larger than the ones focussed on angiogenesis. Moreover, we preferred to limit our comments of immunotherapy on those aspects that seemed to be particularly involved in an interaction with angiogenesis. Nevertheless, we have broadened this section and have especially referred to the combinations in other cancers. Thank you for the suggestion.

- “Regarding the comment on the clinical trial sponsored by the GEM, it would be good if the authors could include more details of the study,... centers involved, end-point, some background on durvalumab and cediranib.”

To answer this point, we have added information on the study endpoints, the centers involved and the number of patients we expect to include in the following manner: “The Grupo Español de Melanoma (GEM), a Spanish collaborative group, has recently designed a phase II, single-arm study in patients with metastatic UM who will be treated with the combination of durvalumab, an antiPD-L1 inhibitor, and cediranib, a multikinase inhibitor of VEGFR, PDGFRβ, KIT, FLT-1 and -2. The primary endpoint is to evaluate the efficacy and response rate of the combination of Cediranib and Durvalumab in patients with metastatic UM with biopsiable disease at baseline, at first line or after failure to first line systemic or liver directed therapies. Five centers will be involved, and 18 patients are expected to be included, although the total number of patients may increase to 27 if the ORR > 20%.”

Reviewer 2

- “Figure 4. organization of the text shoul be improve, The title is not really good for Figure 4.”

We improved the comprehension of the figure caption in the following manner: “Comparison of immunogenicity between UM and cutaneous melanoma; Data extracted from the TCGA database. (A and B) Less than 25% of UM tumors express high CD8A and PDL1, whereas
close to 50% of cutaneous melanoma tumor samples express high levels of both genes. (C) Kaplan-Meier PFS curves of UM and cutaneous melanoma with the highest (red) and lowest (blue) quartile of expression of both CD8A and PDL1. Highly immunogenic tumors are seem to have better prognosis in cutaneous melanoma, although the opposite effect is seen in UM, even showing that all patients in the CD8A\textsuperscript{high}/PDL1\textsuperscript{high}-quartile experience disease relapse.

**Reviewer 3**

- “One should also include less recent work, and any ref to the role of macrophages in vessel development and the genetic basis of inflammation in uveal melanoma is missing and should be added. The work of the groups of Grossniklaus, Kivela, Cree and Jager on angiogenesis is lacking and should be added.”

We apologize for leaving out the role of macrophages, especially M2 infiltrating cells. We have added references to the genetic basis of inflammation and to the role of macrophages in angiogenesis and their prognostic significance: “Finally, apart from endothelial cells and pericytes, macrophages seem to be major contributors to angiogenesis. In 2001, tumour infiltrating macrophages (TAMs) were found to be associated to worse prognosis in UM [53], and Bronkhorst et al demonstrated in 2010 that this was mainly due to polarized M2 macrophages [54]. These macrophages are associated to immunosuppressive functions and are proangiogenic cells [55,56]. Interestingly, Bronkhorst et al found that a high infiltration of M2 macrophages was correlated to monosomy 3 and increased microvascular density in UM [54]. Additionally, macrophages present in choroidal neovascularization specimens express VEGF, which further supports a role of these cells in UM angiogenesis [57].”

- “And please clarify everywhere about which tumor you are writing: uveal melanoma, cutaneous melanoma, or other. Much of the work on vascular mimicry was NOT done on UM.”

We apologize for this lack of clarity, which can prove misleading. We do recognize that not all of these works were specifically carried out in uveal melanoma models, and not all of the given information can be generalized to uveal melanoma. However, we do believe that the molecular findings of other tumours can contribute to a better global understanding of vasculogenic mimicry, despite the major limitations in extrapolating these results. We therefore completely agree to the importance of specifying the type of tumours in which each work was carried out, so the information is not misleading. Thank you for pointing this out.

- “I am not sure there is any data that show that vasculogenic mimicry is essential. It may only be a sign of high tumor aggressiveness, corresponding with loss of chromosome 3/class 2 tumors.”

Thank you for pointing this out. We have changed the abstract and added a more accurate sentence. We will further expand this in the text: “Vasculogenic mimicry, the ability of melanomas to generate vascular channels independently of endothelial cells, could play an important role, but no effective therapy targeting this process has been developed so far.”

- “Line 43; not Norway or Denmark. Choose one or say: and.”

We have changed this to Norway and Denmark.
- “Line 49: name BAP1 as the last one, not the first. M3 and 8q were discovered much earlier.”
We have changed the order of the sentence as you suggest.

- “Line 63; antibody instead of antibodies”
We have changed this as suggested.

- “Line 65: behind durabele complete… a word is missing”
We have added the missing word “responses”.

- “68: after CTLA-40 the word monoclonal antibody is missing”
We have added the term monoclonal antibody, as suggested.

- “Lines 69 and 72: can you illustrate what you mean with 21 and 50%?”
We have clarified the term plateau with the following sentence after 21%: “implying that one fifth of patients could achieve long term survival and could eventually be cured with immunotherapy alone”.

- Line 109: “There are many papers on the prognostic role of angiogenesis in uveal melanoma, and also about the role of macrophages that play a role in vessel development in uveal melanoma:
We apologize for missing this information. We have changed the sentence and added the references you suggest. We have added the reference to TIMs in the corresponding section referring to the role of macrophages in angiogenesis.

- 115: please explain the signature in the text. In fig 1, you name it the angiogenesis enrichment score, bit how the score works should be added to the paper. Which genes are used?
We have added the following text to explain the signature: “The “BIOCARTA_VEGF_PATHWAY” signature is compounded of 29 different genes related to angiogenesis, including HIF1A, the eukaryotic translation initiation factor (EIF), VEGFA and von Hippel Lindau (VHL), amongst others.

- **146; does NOTCH 1 play a role in uveal melanoma angiogenesis?**

There is no solid evidence of the role of Notch1 in uveal melanomas. Nevertheless, hypoxia does have a role in the activation of Notch, and this subsequently results in tumour growth and invasion. In order to clarify this point, we have added the following text with the corresponding references: “In UM, however, there is no established direct relationship between the Notch signalling pathway and angiogenesis. Nevertheless, hypoxia does seem to promote growth and invasion of uveal melanoma cell lines through the activation of Notch and MAPK”.

- **164-189 Please make it clear which of this work has been done in uveal melanoma and which work in other tumors.**

We have specified the different cell lines and types of tumours in which each study focused on.

- **191: which melanomas?**

We have specified the type of melanoma in which each work was carried out in order to provide clearer information.

- **The statement in line 206 that in uveal melanoma, vasculogenic mimicry may play a predominant role in early stages is contradicted by the finding, that especially more malignant, larger, class 2 tumors have vasc mimicry (paper Maniotis and Harbour).**

This is an interesting point and have added this to the discussion in this paragraph in the following manner: “Therefore, vasculogenic mimicry seems to play a predominant role in early stages of disease development, both in cutaneous and UM. These observations seem to be contradicted by the initial findings of Maniotis et al and Chang et al where larger tumours seemed to be richer in matrix-embedded channels. Whether vasculogenic mimicry is a time-dependent event in disease progression or simply identifies inherently more aggressive tumours is unclear.”

- **“Line 208 that the highest OS rates have been reached with anti-angiogenics is an over statement: the number s of patients are so low, that no such statement can be made.”**

We apologize for including this sentence. We have deleted it as we acknowledge this was probably an overstatement.

- **Line 224 should state the group sizes.**

We have added the following line to reflect the small number of patients included in these trials, and refer the reader to the table for further detail of these trials: “Moreover, one must bear in
mind that the number of patients included in these trials is low, with less than 20 patients in most cases, which hinders the generalizability of the data (see table 1)."

- Line 234 is also an overstatement to which hardly anyone would agree. When one looks at mPFD and ORR, the comment has to be changed.

We acknowledge this statement is conflicting, so we have changed it to: “Therefore, new approaches are needed in order to optimize outcomes with antiangiogenic therapies in UM.”

- The statement in line 243 that vas mimicry has been discussed and is essential, is NOT true. I see no evidence anywhere that it is essential.

You are right, we apologize for this mistake. The statement is wrong. We do believe that targeting vasculogenic mimicry could be beneficial, so we have changed it to the following: “However, as we have discussed, vasculogenic mimicry seems to be present in more aggressive melanomas, and although its biological implication is unclear, its inhibition could have a potential therapeutic role”.

- 249: which of these models used Uveal melanoma? From the text, it is not clear which melanoma was used, and which animal. Please clarify the texts.

We have specified what models, cell lines and animals were used in every molecule tested.

- Line 292 and elsewhere: immunosuppressive (2 ps!) Also line 299.

We have corrected both grammatical mistakes.

- Line 292 add references to infiltrate

We have added this reference, as suggested.

- Line 293: this, not dis. (paragraph 4 should be checked for English)

We have corrected the English grammar in this paragraph in order to improve its comprehension.

- Fig 4C: have you not exchanged UM and CM? UM usually die within 20 mths when highly inflamed.

We are not sure we understand this statement. We have revised the plot and they are not exchanged. The median PFS of highly inflamed UM is about 17 months in this KM plot, we do not have overall survival data.
- 317: the lack of lymphocytes in UM is genetically determined. Please use this information:

We have added this citation with the following sentence: “The immune infiltrate in UM seems to be related to genetic alterations, with the lack of BAP1 mutations showing a richer T-cell infiltration”.

- 327-333: it seems all of this refers to cutaneous melanoma. Please clarify.

We have clarified these paragraphs by specifying that this information referred to cutaneous melanoma.

- 340-349: it is here also not clear from which malignancies you get the statements.

We have specified the malignancies in which each work was carried out.

- Line 359: where do you get the statement from that UM is more dependent on angiogenesis than cutaneous melanoma?

We acknowledge the sentence is confusing. We referred to the worst prognosis conferred to UM angiogenic-rich tumours, which does not seem to be as evident in cutaneous melanoma according to the data we present. We have therefore changed this sentence to: “Despite angiogenesis seems to confer poorer prognosis in UM when compared to cutaneous melanoma(…)

- Line 365 that recruitment is expected to start in the following months should be deleted, as this paper is going to be read in many years to come.

We have deleted this sentence.

- 368: unless you have other statements to build on, change the sentence that vasculogenic mimicry is especially important in this tumour. Also downplay the statement that antiangiogeneic drugs seem to be more effective. I see no proof of that.

We have changed the initial conclusions to the following: “In summary, angiogenesis plays an essential role in the development and progression of UM, and the exact implication of vasculogenic mimicry is still unclear, but could potentially play an important role. The efficacy of antiangiogenic drugs is still clearly insufficient.”

- Do you know these papers?


Thank you for sharing these interesting papers with us. We have added these papers to our review. We have found the second one particularly interesting. We hypothesize that one of the mechanisms that could be involved in the promotion of invasiveness is precisely through vasculogenic mimicry, as has been shown in other tumour types. We have added this comment to the text.