Author’s response to reviews

Title: Blocking podoplanin suppresses growth and pulmonary metastasis of human malignant melanoma

Authors:

Yiming Zhao (zhaoyimingbox@163.com)
Mengqiao Xu (xumengqiaobox@163.com)
Xia Wang (tutu0416@163.com)
Yanfang Pan (yanfang.pan@drug-farm.com)
Xingpeng Zhao (zhaoxing2013@163.com)
Bin Yan (sudayanbin@163.com)
Changgeng Ruan (ruanchanggeng@suda.edu.cn)
Lijun Xia (lijun-xia@omrf.org)

Version: 2 Date: 24 Mar 2019

Author’s response to reviews:

Dr. Linda Gummlich
Editor-in-Chief
BMC Cancer
RE: Manuscript BCAN-D-19-00027R1

Dear Dr. Gummlich,

We are grateful for the opportunity to submit a revised version of our manuscript (BCAN-D-19-00027R1). We appreciated the constructive comments and suggestions of the reviewers, and have revised the manuscript accordingly. Below are our point-by-point responses.

Review 1

1. It is suggested to include a control of parental CHO xenografts for Fig. 3 and Fig. 5 to clearly prove the specificity of SZ168 in vivo.
A. Thanks for the suggestion. We have included parental CHO xenografts as a negative control.

2. The results of Fig. 4 can be removed. The binding activity to melanoma tissues in vivo has been demonstrated in Fig. 7. It also suggested to examine the binding of SZ168 in clinical specimens.

A. Figures 4 show that SZ168 recognizes PDPN in ectopic tumor, which is different from Figure 7. Therefore, we prefer to keep Figure 4. We agree with the reviewer that it is important to examine the binding of SZ168 in clinical specimens. However, we currently do not have access to patient melanoma specimens. We plan to examine clinical samples in the future study.

3. It is also suggested to provide the evidences about that SZ168 inhibits platelet coating on tumor cells or reduces tumor-platelet aggregates in vivo.

A. Thanks for the suggestion. We will include this experiment in our future study.

4. The authors should also provide data about the expression of TGF-b, VEGF-A, PDGF in C8161 xenograft tissues to try to explain the anti-metastatic activity of SZ168.

A. As suggested, we performed a co-culture experiment of C8161 cells and platelets, which are included in Table 1.

5. For better understand the mechanisms of therapeutic effect of SZ168, the authors should consider to use B16F10 mouse melanoma cells inoculation into C57BL6 mice and analyzing the immunological microenvironment within tumors, such as profiles of cytokines or immune cells.

A. The SZ168 is a murine-derived monoclonal antibody, which recognize human but not mouse PDPN. Thus, it is not feasible to use B16F10 mouse melanoma cells.

Review 2

1. In Figure 1. From the Western blot depicted in Fig 1 C and D there are two bands (25 and 36 KDa) that correspond to PDPN. Can the authors elaborate on this. Are there two isoforms of the protein?

A. PNPN is a mucin-type O-glycoprotein. The two bands (25 and 36 kDa) indicate different glycosylated form of PDPN. SZ168 recognized the fully-glycosylated PDPN, not the peptide backbone, suggesting that its epitope is glycosylation-dependent. The interaction of PDPN and platelet CLEC-2 is glycosylation-dependent. Therefore, SZ168 does not reduce the platelet aggregation rate is less likely caused by its inability to recognize under-glycosylated PDPN.
2. Other question is relative to the fact that the antibody SZ163 is not able to recognize the 25KDA PDPN band. Why is that? Could this be somehow related to the fact that this antibody is not able to reduce the platelet aggregation rate?

A. The Thr52 in PLAG3 region of PDPN is a key site for O-glycan sialylation, which is essential for binding to platelet CLEC-2 to induce platelet aggregation. SZ163 may not recognize Thr52 and thus it cannot inhibit tumor cell-induced platelet aggregation.

3. Finally, the authors should explain why they have used EPR7072 as a control as it only recognizes the 25KDa band.

A. EPR7072 (a monoclonal antibody identified human podoplanin that purchased from Abcam) was the only anti-human PDPN mAb we had. Therefore, we used it as a control.

4. In Figure 4A it should be better depicted that the second panel is a zoom of the tumors or metastasis shown in the first panel. Also, it should be at least stated form which animals are this primary tumors shown. I believe that form the CHO/hPDPN + Mouse IgG. Fig. 7A. It should be better shown or explained that the second panel is a zoom of the metastasis shown in the first panel

A. As suggested, we have revised Figures 4 and 7.

5. Typos: in the Methods section of the abstract it should be read "… inoculating subcutaneously human malignant…"

A. We have corrected all typos.

We believe that the revisions, suggested by the reviewers, have greatly improved our manuscript. We have also performed additional English editing to improve the presentation of our manuscript. We hope that our manuscript is now acceptable for publication in the BMC Cancer.

Sincerely,

Yiming Zhao

Jiangsu Institute of Hematology
The First Affiliated Hospital of Soochow University
188 Shizi Street, Suzhou, Jiangsu Province, China, 215006
Tel: 086 512 67781379, Fax: 086 512 65113556
E-mail: zhaoyimingbox@163.com