Dear editor and reviewers,

So thanks for your kindly consideration and precious suggestions on our revised manuscript "**Clinical observation of apatinib mesylate for the treatment of multiple micrometastases in the brain: report of 2 cases**". The manuscript has been revised seriously according to the kind suggestions of reviewer, all questions raised by the reviewer have been addressed and the point to point explanation of their questions have been listed following. Thank you for your kindly consideration. Please do not hesitate to contact us if any further information about this paper is needed.

Thank you for your kind considerations!

Best regards,

Jun-Hui Guo

Responses to Reviewer's Comments

a) Introduction and discussion should be edited for adding mechanisms of action (beyond VEGF)

Our response:

Thanks very much for your kind consideration and suggestions. We have been edited for adding mechanisms of action in the Introduction and discussion part. Please check it. The specific additions are discussed below:

1.The new content of the mechanism in the Introduction as follows：

Page 2：

Apatinib is a small-molecule multitargeted tyrosine kinase inhibitor(TKI) that highly selectively binds and inhibits VEGFR-2 activity, blocks VEGFR-2 binding to VEGF, and blocks VEGF/VEGFR-2 mediated signal transduction by restraining

several signaling pathways: the Raf/MEK/Erk pathway, the p38-MAPK pathway and the PI3K/AKT/mTOR pathway,[12]

Page 2，3：

Apatinib has demonstrated encouraging anti-tumor activities and tolerable toxicities in several solid tumors, including lung carcinomas, breast cancer, hepatocellular carcinoma and osteosarcoma cancers, etc.[14] And apatinib inhibits glycolysis by suppressing the VEGFR2/AKT1/SOX5/GLUT4 signaling pathway in ovarian cancer cells.[15] Furthermore, apatinib inhibits the function of ABCB1 in certain cancers, reverse ABCG2(BCRP/MXR/ABCP)-and Pglycoprotein (ABCB1/MDR1)-mediated multidrug resistance.[16,17]

All the modifications are shown in purple text in the manuscript (Page 2,3).

2.The new content of the mechanism in the discussion as follows：

Page 10： It has been reported that apatinib effectively suppressed cell proliferation by regulating glycolysis through the VEGFR2/AKT1/GSK3β/SOX5 signaling pathway in ovarian cancer cells.[25] Preclinical findings have indicated that apatinib also appeared to reverse multidrug resistance by inhibiting the transport function of multidrug resistance protein 1(ABCB1), multidrug resistance-associated protein 1(MRP1, ABCC1), and breast cancer resistance protein (BCRP, ABCG2).[38,39] Apatinib has been found to repressed the expression of STAT3 and BCL-2 and suppressed the growth of osteosarcoma.[40]

All the modifications are shown in purple text in the manuscript (Page 10).

b) Discussion is poor. Why the effect in this two cancer types? Previously apatinib had been mentioned active in gastrointestinal types. Could you suggest putative actions for limiting tumor development?

Our response:

Thanks very much for your kind consideration. Regarding your question, we explain as follows:

Apatinib has exhibited antitumor effects in a variety of solid tumors. But for intracranial tumors, we have only observed the effect in this two cancer types. At

present, the situation in other cancer types has not been observed, we will continue to observe in the future.

c) Why the used posology? If as you sentenced...There is no clear recommendation or guidance for the study of studying the initial dose and or maintenance dose...

Our response:

Thanks very much for your kind consideration. Regarding your question, we explain as follows:

In both cases, the initial dose of apatinib was 250mg.po.qd, which was increased to 500mg.po.qd according to the patient's physical condition. However, after the increase, adverse reactions such as fatigue, anorexia, and significant increase in blood pressure occurred, and then returned to 250mg.po.qd treatment. Apatinib 250mg.po.qd is recommended for the treatment of such patients.

d) The traditional medicine or other common intervention could be shared for these two patients?

Our response:

Thanks very much for your kind consideration. About “The traditional medicine or other common intervention could be shared for these two patients?” We explain as follows:

At present, the main treatments for brain metastases include surgery, whole brain radiation therapy (WBRT), stereotactic radiosurgery (SRS), and chemotherapy. However, the first patient with esophageal cancer, 82 years old, was older and had more severe bone marrow suppression using chemotherapy. Therefore, chemotherapy could not be tolerated. The second patient with cervical cancer, 40 years old, although young, also had more severe bone marrow suppression using chemotherapy. Therefore, chemotherapy was also not tolerated.

In addition, the intracranial lesions in both cases were small, so radiotherapy was not considered.

Therefore, these two patients have no other better traditional medicine or other common intervention.

e) **Some sentences in discussion lack references supporting them.**

Our response:

Thanks very much for your kind consideration and suggestions. We have added corresponding references to support some sentences in discussion in our manuscript. Please check it. The specific additions are discussed below:

Angiogenesis is closely related to the occurrence, development and metastasis of malignant tumors.[27] （H anahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013])

Tumor cells produce a variety of substances that lead to angiogenesis, which provides essential nutrients (including oxygen and nutrients, etc.) for the further growth of tumors and the excretion of metabolites.[28] (Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017;20(4):409–426 [PMID: 28660302 DOI: 10.1007/s10456-017-9562-9]）

It is found that stromal cells in tumor microenvironment play an important role in promoting tumor invasion and metastasis.[30] （Denton AE, Roberts EW, Fearon DT. Stromal Cells in the Tumor Microenvironment. Adv Exp Med Biol. 2018;1060:99–114 [PMID: 30155624 DOI: 10.1007/978-3-319-78127-3_6]）

VEGF-C is a mitogen directed against lymphatic endothelial cells and has a dual role in stimulating blood vessel and lymphangiogenesis.[32] （Yonemura Y, Endo Y, Fujita H, et al. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. Clin Cancer Res. 1999;5(7):1823–1829 [PMID: 10430087]）

Preclinical findings have indicated that apatinib also appeared to reverse multidrug resistance by inhibiting the transport function of multidrug resistance protein 1(ABCB1), multidrug resistance-associated protein 1(MRP1, ABCC1), and breast cancer resistance protein (BCRP, ABCG2).[38,39]（38.Tong XZ, Wang F, Liang S, et al. Apatinib (YN968D1) enhances the efficacy of conventional chemotherapeutical drugs in side population cells and ABCB1-overexpressing leukemia cells. Biochem Pharmacol. 2012;83(5):586–597 [PMID: 22212563 DOI: 10.1016/j.bcp.2011.12.007]; 39. Mi YJ, Liang YJ, Huang HB, et al. Apatinib (YN968D1) reverses multidrug resistance by inhibiting the efflux function of multiple ATP-binding cassette transporters. Cancer Res. 2010;70(20):7981–7991 [PMID: 20876799 DOI: 10.1158/0008-5472.CAN-10-0111]）

Apatinib has been found to repressed the expression of STAT3 and BCL-2 and suppressed the growth of osteosarcoma.[40] （Liu K, Ren T, Huang Y, et al. Apatinib promotes autophagy and apoptosis through VEGFR2/STAT3/BCL-2 signaling in osteosarcoma. Cell Death Dis. 2017;8(8):e3015 [PMID: 28837148 DOI: 10.1038/cddis.2017.422]）

All the modifications are shown in purple text in the references in the manuscript (Page 14,15).