Reviewer's report

**Title:** Effect of troglitazone on tumor growth and pulmonary metastasis development of the mouse osteosarcoma cell line LM8

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**Reviewer:** Tong-Chuan He

**Reviewer's report:**

In this report, Aizawa J et al. investigated whether troglitazone (TGZ) can function as possible therapeutics in the treatment of growth and metastasis of osteosarcoma. The authors used mouse osteosarcoma line LM8 and treated the cells various doses of TGZ to analyze cell proliferation, and cell invasion and motility. The effect of TGZ on Akt signaling was also assessed by Western blot analysis of Akt and p-Akt. The authors found that TGZ inhibited cell proliferation, and that TGZ-treated cells were less invasive and less motile than untreated cells. TGZ treatment inhibited MMP-2 activity, and decreased the level of p-Akt without affecting Akt expression. In subcutaneous implantation assays, treatment of tumor-bearing mice with TGZ decreased the expression and activity of MMP-2 within the tumor, and inhibited primary tumor growth and pulmonary metastasis development. Based these findings, the authors concluded that inhibition of Akt signaling by TGZ may decrease the secretion of MMP-2, resulting in the decrease of invasiveness and motility in LM8 cells. Thus, TGZ may offer a new approach in chemotherapy for osteosarcoma.

Although not completely novel, the reported findings were interesting and intriguing. The manuscript was well written. For the most part, the conclusion was supported by the experimental findings. There was some novelty in the studies as the authors examined the involvement of Akt signaling pathway in TGZ’s anti-tumor activity. However, the reported studies can be further improved if the following issues can be effectively addressed.

**Major Compulsory Revisions**

1) In Figure 1A, the authors tried to demonstrate the TGZ effect on LM8 cells. However, it’s hard to interpret the data as it is unknown what underlies the morphological changes. More rationales and justifications have been provided.

2) TGZ is known to promote differentiation in a non-specific fashion. The authors ought to demonstrate if TGZ induces osteogenic markers, such as alkaline phosphatase, osteocalcin and osteopontin in vitro and in tumors in vivo.

3) The authors tried to use PPARgamma antagonist GW9662 to counter TGZ effect on LM8 cells. However, it was reported that GW9662 can actually inhibit breast cancer growth independent of PPARgamma activation [Br J Pharmacol. 2004 December; 143(8): 933–937]. To effectively encounter TGZ effect, the authors should consider the use of PPARgamma silencing approach to
demonstrate the role of PPARgamma in TGZ anti-osteosarcoma activity.

4) The authors determined the role of TZD on Akt phosphorylation. While the results were intriguing, the mechanism is totally unknown and not explored by the authors. Would Akt silencing render the cells more sensitive or resistant to TGZ treatment?

5) Without providing any mechanistic connections, the authors examined the inhibitory effects of TGZ on MMP2, VEGF, and CD34 expression. The authors ought to provide evidence and/or rationale why these genes were chosen or how they are regulated.

Minor Essential Revisions

1) The authors administered the TGZ in drinking water using ethanol as solvent. This is different from the food delivery approach reported by Bruce Spiegelman’s group. It was not clear how stable the drug was under this condition. It’s also unclear how the dosage was determined.

Discretionary Revisions

1) In the animal studies, the authors used ether anesthesia. Ether has not been recommended for small animal surgeries. Please consult with the institutional animal use and care committee. The subcutaneous injection only produces momentary pain so anesthetics may be avoided.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.