Reviewer's report

Title: Validation of a VEGFR2 antagonist peptoid in vivo

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Reviewer: Bjorn Olsen

Reviewer's report:

(1) This manuscript describes a striking delay in MMTV-PyMT tumor progression in mice given a combination of doxorubicin and the peptoid GU81. GU81 is a derivative of the peptoid GU40C4 that has shown promising antiangiogenic activity. The binding of GU81 to the VEGFR2 receptor is improved compared with GU40C4 and the authors have recently reported that treatment of mice carrying 4T1 highly metastatic breast cancer tumors with GU81 as a single agent results in some reduction in mean tumor volume but relatively insignificant effects on tumor vessel density.

(2) In the present study, the authors describe experiments showing that GU81 has no effect on MMTV-PyMT tumor volume or tumor weight. They also report (data not shown) that administration of GU81 alone, doxorubicin alone, or GU81 in combination with doxorubicin had no effect on microvessel density, despite the clear inhibitory effect of GU81 on VEGF-dependent stimulation of VEGFR2 phosphorylation with endothelial cells in vitro. Surprisingly, tumors from GU81 treated animals appeared to have increased levels of VEGF in the tumor compared to control-treated animals. Furthermore, this effect of GU81 on VEGF levels was abrogated when GU81 was combined with doxorubicin.

Major Compulsory Revisions:

(3) The insights gained from this study are not clear; a deeper analysis of the mechanistic basis for the in vivo effects of GU81 and the additive effects of doxorubicin is required.

(4) The title of the study needs to be changed. The current title, “Validation of a VEGFR2 antagonist peptoid in vivo”, is misleading in that the use of the peptoid for treatment of tumors in vivo has already been published (PLoS One 2009). The only new in vivo validation aspect of GU81 in the present study is that for the MMTV-PyMT breast cancer model it does not work when administered alone. Results from in vitro experiments show that it represses VEGFR2 phosphorylation in endothelial cells but no data are presented to indicate that such repression is the primary action of Gu81 in vivo. In fact, the increased levels of VEGF and lack of effects on tumor vessel density would be consistent with the hypothesis that GU81 has no suppressive effects on VEGFR2 phosphorylation in vivo.

(5) While the data in this study are intriguing, they leave open the question of what the mechanism of action of GU81 on tumor vasculature or tumor cells may
be in vivo. The authors assume that it acts by blocking activation of VEGF receptors R1 and R2, but based on the absence of any effect on vessel density and the increased levels of VEGF in the tumor as a result of treatment with the peptoid, one wonders whether the compound has other effects on cells that may be unrelated to suppression of VEGFR signaling. Additional mechanistic data to explain the ability of GU81 to stimulate VEGF levels in the tumor are required.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I DECLARE THAT I HAVE NO COMPETING INTERESTS