Reviewer’s report

Title: The endogenous soluble VEGF Receptor-2 isoform suppresses lymph node and lung metastases in a mouse immunocompetent mammary cancer model

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Reviewer: Bronislaw Pytowski

Reviewer’s report:

sVEGFR-2 was recently described (Albuquerque et al. Nature medicine 15:1023, 2009) by the laboratory of Jayakrishna Ambati who is a co-author on the present manuscript. The biology of sVEGFR-2 is perplexing and at this time poorly understood. Paradoxically, SVEGFR-2 is reported to bind to and act as a biological sink specifically for VEGF-C and not VEGF-A. This is in contrast to the transmembrane form of VEGFR-2 that binds to VEGF-A with much higher affinity then to VEGF-C.

In the present manuscript, Shibata et al. provide evidence that soluble VEGF receptor-2 (sVEGFR-2) suppresses lymph node and lung metastasis in a mouse model of breast cancer. The basic finding is expected since numerous studies have shown that inhibition of the VEGF-C/VEGFR-3 axis reduces metastasis to lymph nodes. However, such activity by a novel endogenous protein (sVEGFR-2) is of interest and the manuscript should be considered for publication if the authors address the comments below.

Major revisions or comments from authors required:

1. The authors use intratumoral injection of an expression vector for sVEGFR-2 into syngeneic breast tumors followed by electrotransfer. Why was thus manner of administration chosen? The authors should comment on attempts to express and purify sVEGFR-3 and why administration of purified sVEGFR-3 was not possible. The use of soluble proteins has two major advantages over gene therapy: a). it parallels much more closely possible therapeutic use of a protein such as sVEGFR-2 and b) it allows for dose-response studies.

2. The authors should attempt to measure the amount of sVEGFR-2 protein present in the circulation of the mice. This would help to explain the mechanism of reduction in the number of metastatic foci in lymph nodes and lungs by sVEGFR-2. i.e. is sVEGFR-2 acting solely on the primary tumor to reduce lymphangiogenesis and consequent metastasis or is it possible that sVEGFR-2 acts also directly on the metastatic nodules.

3. One of the most confusing aspects of the manuscript is the choice of endostatin as one of the treatment groups. The authors need to explain in the introduction what the reason for this choice was. Was it a positive control? A much more commonly used molecule in studies of lymphatic metastasis is
soluble VEGFR-3 that acts as trap for VEGF-C (see for example He et al. JNCI, 94:819, 2002). Published results on anti-lymphangiogenic effects of endostatin are sparse. The authors cite their own paper (Shibata et al. Cancer gene Ther 14:268, 2007) while neglecting to cite another study with similar results (Birdau et al., Cancer Res 67:11528, 2007). This should be corrected.

4. In all the reported results, gene therapy with endostatin is considerably more potent than that with sVEGFR-2. Yet the authors barely mention this difference in the Results section (for example, description of Figure 1C, page 10, top). The authors should expand the section of the Discussion that describes the results with endostatin and explain how these results relate to those obtained with sVEGFR-2.

Minor essential revisions or comments from the authors:

1. All histology figures require bars indicating magnification.

2. The histological method to determine p53 mutation in the tumors (Figure 2D) needs to be added to Materials and Methods. Why was this method chosen as opposed to sequencing of the p53 gene from the implanted tumor cells?

3. Since the implanted cells were labeled with luciferase, did the authors compare total metastatic load in the lymph nodes and the lungs using luciferase activity. Such measurements would greatly strengthen those reported in Figure 3.

4. The authors state that in legend to figure 5F that apoptotic tumor cells are frequently observed along blood vessels. How are the vessels identified as blood vessels? Why would there be more apoptotic cells near blood vessels (typically, the opposite is observed)?

5. Table 1 should be expanded to list specific sites of metastasis. The results should be discussed in relation to what is known about breast cancer metastasis in other experimental models and in humans.

6. The authors cite their own study (ref 24, page 14, top) regarding blocking of VEGFR-3 with antagonist antibodies. Studies from other laboratories should be cited. (For example: Roberts et al. Cancer Research 2006, 66:2650, 2006; Hoshida et al. Cancer Res. 66:8065, 2006)

7. Mention in the discussion that one explanation for reduced metastasis in sVEGFR-2 and Endo groups is that the primary tumors were smaller (especially pEndo).

Minor comments:

1. In Methods under Cell line and animals, BJMC3879 should be identified as a mouse cell line.

2. The sentence on page 8 starting with “The mammary carcinoma tissues…” is cumbersome and should be revised.

3. In figure legend to Figure 1C, the statement “…pEndo groups begun to
decrease significantly” should be changed to “rate of growth of the tumors in pEndo group was decreased.”. There is no decrease in tumor volumes in this figure.

4. In figure legend 2, the sentence “Fewer lymph nodes with metastasis..” should be removed as it does not apply to this figure.

5. Consider moving figure 3 to the end.

6. On page 4, in the sentence containing “…whether it would serve as a therapeutic target…” the word “target” should be changed to something

**Which journal?:** Not appropriate for BMC Medicine: an article whose findings are important to those with closely related interests and more suited to BMC Cancer

**What next?:** Offer publication in BMC Cancer after minor essential revisions

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

Regarding points 1-3, I am an employee of ImClone Systems, a wholly owned subsidiary of Eli Lilly and Company. I am a project leader in development of a monoclonal antibody therapeutic with activity similar to that of the compound described in the manuscript.