Reviewer's report

**Title:** Collagen induced arthritis increases secondary metastasis in MMTV-PyV MT mouse model of mammary cancer

**Version:** 4  **Date:** 6 June 2011

**Reviewer:** Lesley Ellies

**Reviewer's report:**

Major Revisions:
1) The authors have not addressed the fact that epidemiologic studies indicate that women with arthritis are protected from the development of breast cancer. In the second paragraph of the introduction, the authors cite Askling et al. 2005 as evidence of statistically significant risk ratios between autoimmune arthritis (AA) and various malignancies including breast cancer. While the statement is correct, the implication is that inflammation increases the risk for these cancers. However the data from this study indicate a 20% decrease in risk for breast cancer in the AA cohort. These data do not invalidate the current study since those patients who do have breast cancer and AA may be at higher risk for metastasis. The hypothesis needs to be reframed in this context.

2) Fig 1.D ii. This H&E section appears to be an area of hyperplasia adjacent to a lymph node rather than an area of solid tumor with immune cell infiltration. Please check this section. Furthermore, there is no obvious inflammation apparent in Fig 1.D iii. This raises concern regarding the authors’ statement that inflammation in the tumor microenvironment was determined by quantitating the H&E stained sections. The presentation of these data is confusing as the authors appear to have the expertise to out this analysis. A more appropriate approach would be to quantitate immune cells as identified by immunostaining – typically F4/80 staining to assess infiltrating macrophages.

3) While lung metastases are the usual site of metastasis in the MMTV-PyV MT model, bone metastases are not. The current findings are important in this respect as the authors mention, since bone is one of the sites for human breast cancer metastasis. However the data from the radiographs is not definitive and it is difficult to see clear osteolysis at the magnification presented. To clearly present the bone findings as PyV MT metastases, histological sections need to be stained with anti-PyV MT antibodies or anti-cytokeratin antibodies to show the presence of an epithelial tumor mass at this site. See Maglione JE, et. al. Polyomavirus middle T-induced mammary intraepithelial neoplasia outgrowths: single origin, divergent evolution, and multiple outcomes. Mol Cancer Ther. 2004 Aug;3(8):941-53 for relevant antibodies.

Minor Revisions:
1) Tables 1 and 2 should include some measure of variability for the integrated
density.

2) Table 2. Please define NaN.

3) Figure 3C. The sections as presented are at different magnifications. The sections should be at the same magnification and the magnification should be indicated in the figure or in the figure legend.

4) Figure 4. C. Figures i-iii are repeated from Figure 3. It would be appropriate to use different figures to indicate the use of multiple mice in the studies. The figure legend states that the H&E sections of joints and lungs are at 200x, however the sections of joints are clearly at a different (lower) magnification than the lungs. Photomicrographs within figure 4A also appear to be at different magnifications.

5) Figures 5-7. Significant differences have not been clearly described in the figures or figure legends. Certain significant differences have been marked on the graphs, however it appears that more bars show significant differences than have been indicated on the figures or in the figure legends. The authors may be focusing their analysis on particular groups, but the way the figure legends are written it suggests that the only significant differences are in these groups when the data on the graphs suggests otherwise. The authors should consider using horizontal bars to indicate the groups that are significantly different from one another as a means to clarify the graphs.

Discretionary Revisions

1) Introduction last paragraph: secondary metastasis - secondary is redundant since a metastasis by definition is a secondary site of tumor formation.

2) Materials and Methods:
Mice: The second sentence is not clear as written. This should be clarified e.g. The PyV MT mice were maintained as heterozygotes and backcrossed from the original FVB/N strain into the C57Bl/6 strain until congenic.
Sentence 3 “Approximately 50% ....” is unnecessary and should be deleted. Details of the PCR amplification are standard, previously published as cited and unnecessary. They can be deleted.

3) Measurement of cytokines: Details of the procedure can be compressed, since the protocol follows the manufacturer’s instructions. Detection and normalization procedures are important to retain. Histology and Image analysis sections can be compressed.

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

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:**

I declare that I have no competing interests