Reviewer's report

**Title:** High infiltration of mast cells predicts worse outcome following resection of colorectal liver metastases

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**Reviewer:** Jean S Marshall

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In this manuscript the authors examine the relationship between peritumoral infiltrates of immune cells and long term survival in subjects undergoing liver resection for liver metastasis in colorectal cancer. This manuscript raises some interesting points but further clarification of a number of issues would be useful for appropriate interpretation.

1. The authors have used formalin fixed tissue for their study. It is well recognized that formalin is not the optimal fixative for human mast cell visualization as well defined in the older literature. Was microwave antigen retrieval used to enhance the ability to detect mast cells using tryptase immunohistochemistry?

2. No indication of controls for immunohistochemistry were given, what number of cells stained positively using isotype control antibodies?

3. More information on the specific cell counting strategy used needs to be provided within the manuscript. Although several papers are referred to for methodology, the strategies used in these references are not identical. How were tumoral and peritumoral areas defined and what area was evaluated for cell numbers (ie mast cell per 10 fields, or for a given number of microns squared)

4. Tryptase staining alone is not appropriate to define mast cell numbers. It is well recognized that tryptase can be taken up by neighboring cells and that macrophages that phagocytose apoptotic mast cells can then stain positively for tryptase. C-Kit staining, FcER1 staining or Toluidine blue, would be excellent strategies to confirm findings with tryptase, alternatively pre-staining with Alcian blue at low pH and tryptase immunohistochemistry (as a double stain) could be useful to confirm that the cells being counted are mast cells.

5. Given the existing data that shows a close correlation between microvessel density and mast cells and the known link between highly angiogenic tumors and poor long term disease outcome the authors need to better justify the use of mast cells rather than angiogenic indicators as an approach to predicting survival. Are mast cell counts simply a surrogate marker for vessels?