Responses to reviewer's comments:

Manuscript title:

Plasma matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-7 (MMP7) levels are significantly elevated during the first month after colorectal resection surgery which may promote the growth of residual metastases (manuscript NO: 62917).

Reviewer #1:

Specific Comments to Authors: Congratulation to the author for their track record in study the role of protein that are proangiogenic in colorectal cancer. This paper as therapeutic impactions for the timing of chemotherapy and perhaps for development of drugs that could target this MMP proteins in the post -operative phase or perhaps, colorectal surgeon may change to alternative anti-cancer drug such the immunotherapy agent. However, I have a few comments that I believe the author may like re-examine;

Comment 1- I could not find any data showed that a correlation between MMP2 and MMP7 staging of the disease (TNM /pathological staging)

Author's response:

We did look for a correlation of MMP2 and MMP7 plasma levels and pathologic T, N, and overall stage of disease. These analyses were done for the preoperative and the various postoperative time points. The very small Stage 4 group (n=4) obviously limited our ability to assess that group.

As regards MMP-2, no significant correlation was found between MMP2 levels and (T), (N) or pathological stage at 6 of the 7 sampling points. Of unclear significance, MMP2 levels on POD 14-21 correlated with T stage [p=0.002] and with pathological stage [p=0.01]. As regards MMP7, no significant correlation between Preop and postoperative levels and (T), N or pathological sage was found.

The authors also did subgroup analyses as regards lymph node status; MMP-2 and MMP-7 levels at all 7 timepoints were compared between node + and node – patients. As regards MMP-2, at 1/7 time points (POD 14-21) plasma levels were significantly higher in the node negative subgroup. No other significant differences were noted for either MMP-2 or MMP-7 between node positive and node negative groups at the various timepoints. Of note, however, MMP7 levels in the node positive group were 4.5-29.3% elevated (on POD 7-13, POD 21-28, and POD 29-34) vs the node negative results but the differences did not attain significance. The reason for these differences and their import is unclear. A larger study with a more equitable stage distribution would shed light on these issues. The manuscript has been updated.

Comment 2- I know that the authors have demonstrated the correlation of MMP proteins in benign disease and cancer but to further establish the hypothesis that author is putting forward perhaps correlation with patient that have received long chemotherapy/ chemoradiotherapy may give us new insight.

Author's response:

We agree with the reviewer that assessing for differences in pre and postop MMP2 and MMP7 levels in patients who had neoadjuvant chemo or radiotherapy (vs subgroup that did not receive

neoadjuvant treatment) would have value. However as described in inclusion and exclusion criteria we did not include in this study patients who had neoadjuvant treatment in order to best determine the impact of the surgical trauma alone (which was our goal).

As regards the question of colorectal resections for benign vs cancer indications, in a separate study that simultaneously measured perioperative levels of 8 proangiogenic proteins in both the blood and fluid from surgical wounds for up to 3 weeks after MICR (of note, separate benign and malignant groups were included and were analyzed separately), it was noted that wound fluid protein levels were 3-40 times higher than plasma levels which, in turn, were significantly elevated from preop plasma baseline levels. The elevations in both plasma and wound fluid protein levels were similar in the benign and the cancer groups suggesting that indication for surgery (benign vs malignant) does not appear to impact these surgery-related changes.

Comment 3-Emergency patient presenting with obstructing colorectal cancer that undergo laparotomy may have been recruited for longer wound.

Author's response: Authors strongly agreed with comment. Although the authors have considered enrollment of emergent patients into our studies, it proved to be logistically not feasible. Emergent bowel cases often occur in the late evenings or during the night and with little notice. With our current research resources it is not possible to provide staff during evening or night shifts. Also, informed consent at these times in sick patients can be difficult to obtain. Therefore, no emergent cases are included in this study.

Comment 4- I would been more exciting there were correlation between MMP proteins and correlation with KRAS mutation, MIS and MMR.

Author's response:

We agree with the reviewer, however, unfortunately, we do not have a complete data set for these parameters for the study population. The suggested analyses and correlations would be a valuable addition to a future study with a larger and diverse patient population. It is the authors' unsubstantiated opinion, however, that the postop plasma changes we have found reflect the extent of surgical wounding and subsequent healing processes. We do not think that the presence of cancer (or a specific sub type of cancer) drives or is responsible for the noted changes.

Comment 5- Is there a difference in the quantification of MMP protein from frozen plasma and fresh sample

Author's response: There are no studies or literature demonstrating a difference in results between assays done on fresh vs fresh frozen plasma. It is standard practice to quickly process blood samples and then to freeze the plasma or serum immediately. As described in the method section of the paper we collected blood samples, processed them in a timely fashion and then aliquoted the plasma into 0.5 ml aliquots that were stored in cryo-vials at -80^0C until used. Of note, the ELISA kits used in this assay recommend the use of frozen samples. We have strictly avoided repeated freeze-thaw cycles. The frozen plasma samples used in this study had not been previously thawed. Samples were analyzed in 96 well ELISA plates in duplicate and an 8 point duplicated standard curve was included in each assay. The statistical analysis was done by comparison of values between preoperative vs its own post-operative samples. Also, it would

not be possible nor advisable to do separate ELISA's for each patient at each timepoint with fresh unfrozen plasma because we would have to do hundreds upon hundreds of ELISA analyses. Further it would be problematic to compare ELISA results done with different kits on different days (as opposed to analyzing all of each patient's timepoints in a single ELISA).

EDITORIAL OFFICE'S COMMENTS

Science editor:

Comment -1: The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

Author's response: As per comment Power point original figures will be included into the revised version of the manuscript.

Comment -2: The references cited in the text should be put in the square bracket, and then make them be superscript.

Author's response: Reference cited format is updated as per editorial comment.

Company editor-in-chief comment: The title of the manuscript is too long and must be shortened to meet the requirement of the journal (Title: The title should be no more than 18 words).

Author's response: Title of the manuscript is updated as per editorial comment.

Author made best possible title with 19 words as below. Please let us know if editorial still want to shorten the title.

New title: Plasma MMP-2 and MMP-7 levels are elevated for 1 month after surgery and may promote growth of residual metastases.