Peer Review File

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Reviewer A

In this original manuscript from Yang et al., the authors present a retrospective review of patients with gastric cancer who received perioperative chemotherapy or total neoadjuvant therapy (TNT) prior to undergoing surgery at Memorial Sloan Kettering Cancer Center from 2014-2020. The use of TNT in other malignancies (such as rectal cancers) is well described in the literature but has not been well studied in gastric cancer. This approach is of interest as many patients who receive traditional neoadjuvant therapy often do not go on to complete their planned postoperative treatment, which was only ~40% in both the MAGIC and FLOT4 studies. The authors suggest that while this study is limited due to the number of patients included in the TNT group, TNT appears to be safe, feasible, and does not affect surgical morbidity, this is a very well-written manuscript on an interesting topic, and provides important insight based on a single-institutional experience. There are several limitations to the present study which should be addressed, and I suggest major revisions prior to publication:

1. The authors acknowledge a limitation of the study is the small cohort of patients who completed TNT (N = 28). Patients were restaged at 6-8 weeks, and if response was observed, they were continued on 4 months of preoperative chemotherapy. Were the patients who did not have responses excluded from this cohort, and would this impart selection bias for the TNT group?
Reply: The reviewer raises a very good point and ideally, we would have included 'intention to treat' TNT patients. However, patients were retrospectively identified as having received TNT, so these were all patients who had a clinical and/or radiographic/PET response to treatment. We have modified the Methods section to clarify this point (Materials and Methods, 2nd paragraph). We would have expected the exclusion of non-responding patients to impart a favorable selection bias (page 11, lines 3-8), but we instead observed a trend toward worse outcomes among TNT patients. As we

acknowledge in the Discussion section (paragraph 4), there may have been some selection bias toward patients with more locally advanced tumors.

2. One of the major limitations of this study is that the chemotherapy regimens differed significantly between the TNT and perioperative therapy groups, with nearly 80% receiving FLOT in the TNT group vs 31% in the perioperative group. This limitation should be addressed in more detail in the discussion. Additionally, if possible, can a direct comparison be made from the small 28 pt cohort of FLOT TNT matched to the larger cohort of FLOT perioperative?
Reply: Included above in limitations as advised (see page 10, lines 20-22). Included direct comparison of TNT and perioperative patients who received FLOT (see page 9, lines 14-21 and Supplementary figures/tables).

3. It would be helpful to include in the methods section details on how time of recurrence was determined for the recurrence-free survival analysis. Additionally, the RFS rates should be reported in the results section.
Reply: Time of recurrence determined by surveillance scans – included in Methods. Added 24-month RFS rates in Results.
Changes in the text: See Page 5, line 16. See page 9, lines 4-5.

4. It is surprising that in proportion of patients with PET response not statistically significant (page 7, lines 11-12) – is this calculation accurate?
Reply: This was a typo, correction made.
Changes in the text: See Page 8, line 5.

5. 6 total cases with R1 but 3 vs 11% worse with TNT …What are the authors' thoughts on why the positive resection margin rate was higher in the TNT cohort? It may be helpful to provide supplementary data on tumor, operative and pathologic characteristics in patients who had R1 resections.
Reply: As noted in the Discussion (4th paragraph), some clinicians may have chosen more intensified therapy for patients with more locally advanced/aggressive disease. Included supplementary table on patients with R1 resection (see Supplementary Table 1).

6. As there was a higher proportion of patients in the TNT group receiving FLOT compared to the perioperative therapy group, it seems that the TNT group may represent a more contemporary cohort. The authors could consider including a timeline of when these patients were treated to give more context for the reader.
Reply: Included information on when TNT and periop patients were treated. Divided patients before and after 2017, when results of FLOT4 study were presented at ASCO.
Changes in the text: See Results, 2nd paragraph.

7. Median OS and RFS were not reached in either group, which is a limitation that should be addressed in the discussion.
Reply: Median follow up time was 44 months. The majority of recurrences occur within 2-3 years of surgery so we feel that the follow up time was adequate for RFS. Included line addressing follow up time for OS.
Changes in the text: See Page 10, lines 22-23.

8. Why do the authors think the completion of planned chemotherapy cycles was higher in the perioperative group (58%) in comparison to published data from the FLOT4 and MAGIC studies, which was ~40%?
Reply: Patients were not treated on a clinical trial, and there was likely a lower threshold for dose reduction and omission of chemotherapy agents to improve tolerability and allow for completion of planned cycles. Specifically, epirubicin was routinely discontinued in the adjuvant setting.

9. If the data is available, it would be useful to include information on why dose reductions were required in the preoperative setting. The authors suggest that TNT is safe and feasible, though the data in the manuscript (Table 4) only comments on surgical outcomes.
Reply: Included line on reason for dose reductions.
Changes in the text: See Page 7, lines 12-13.

10. What was the survival of the CR patients vs others and what was recurrence pattern?
Reply: None of the CR patients had recurrence. 1 patient passed away for unknown reasons.
Changes in the text: See Page 9, lines 8-9.

11. To be consistent with Tables 1 and 4, it would be helpful to have an "Overall" column for Tables 2 and 3 as well.
Reply: Revised Tables 2 and 3 as advised.

12. I would recommend using the same number of decimal places for each p-value (eg, 3 decimal places).
Reply: P-values adjusted as advised.

13. The authors present data in Figure 2 which suggests that there is no significant difference between survival in patients who received perioperative chemotherapy with or without postoperative therapy. It would be helpful to directly compare the curves of those 2 cohorts with log-rank test (in addition to the analysis of 3 groups together as presented).
Reply: Added log-rank for comparison between the periop patients with and without postop chemotherapy.
Changes in the text: See Figure 2.

Reviewer B

121 patients who received perioperative chemotherapy and 28 who received TNT were analyzed. More TNT patients received FLOT compared to the perioperative group. There was no significant difference in hospital length of stay or surgical morbidity. 14% of TNT patients and 5.8% of perioperative patients achieved a pathologic complete response. There was no significant difference in RFS or overall OS between the two groups. According to the results, the authors concluded that TNT appears to be feasible in a select population, without any increase in surgical morbidity.
Reviewer is very interested in TNT for gastric cancer as well as rectal cancer because many patients undergoing gastrectomy cannot receive postoperative chemotherapy. TNT may increase the total dose of chemotherapy by giving drugs preoperatively when patients have a good general condition. On the other hand, during TNT, tumor may increase the size and make new metastases. Furthermore, a long-time preoperative chemotherapy of TNT may increase postoperative complications. Thus, TNT for gastric cancer still has many unmet issues to be investigated.

A rationale of TNT for gastric cancer using FLOT is unclear. Of course, one of the benefits of TNT is the high compliance of preoperative treatment. However, pCR rate of FLOT is reported to be only 16%. Is this enough to use FLOT as TNT for gastric cancer?
Reply: FLOT is the standard of care perioperative chemotherapy regimen, so we believe this is the appropriate regimen to use for TNT in gastric cancer. The rationale for TNT is not to achieve a pCR (since all patients undergo subsequent surgery) but to improve chemotherapy delivery in the preoperative setting and better treat micro-metastatic disease.

Why did the authors use 3 types of chemotherapeutic regimen? What were indications for each regimen?
Reply: This was a retrospective analysis of gastric cancer patients who received perioperative chemotherapy and underwent surgery. There were 3 main categories of chemotherapy regimen received – 5FU/platinum, 5FU/platinum/epirubicin, and 5FU/platinum/docetaxel. Regimen chosen generally depended on when patient was treated (which regimen was considered standard of care at that time) and performance status (if able to tolerate 3 vs 2 drug regimen).

As the authors mentioned, the study includes a heavy selection bias. Furthermore, it also has a chronological bias. Many patients undergoing TNT received FLOT which was established as a preoperative chemotherapy in 2017 (Albatran JAMA Oncology). Thus, the patients undergoing TNT had been treated more recently, while those undergoing perioperative chemotherapy had been treated more before. Generally, surgical morbidity decreases in recent patients. That can be why the incidences of postoperative complications were comparable between perioperative and TNT patients even though TNT patients received longer preoperative chemotherapy. Moreover, reviewer suspects that perioperative patients mainly underwent open gastrectomy and many TNT patients underwent laparoscopic gastrectomy, although reviewer did not find any explanations regarding types of approach (open or laparoscopic). The high incidence of abscess or wound infection (the deviation of complication types) made reviewer have such a suspicion. The authors should mention the issues described above and discuss these potential biases.
Reply: 4 (14%) TNT patients were treated between 2015 and 2016, and 24 (86%) were treated after 2017 (included in text, see page 6, lines 21-22). A substantial proportion of perioperative patients (45%) were also treated after 2017. In the past 10 years, slightly more patients have undergone minimally invasive surgeries, but the rate of major surgical complications (Clavien-Dindo grade III or higher) appears to be similar in our minimally invasive vs. open cohorts. Of the 28 TNT patients, only 3 (10.7%) underwent laparoscopic surgery (the majority had open procedures). For these reasons, we would not expect the comparable surgical complication/morbidity rates to be attributable to any difference in surgical technique.

Why did so many TNT patients undergo R1 resection (positive cutting stump)? The incidence is incredible. Reviewer is very anxious about the authors' unskillful surgical procedures or inadequate preoperative evaluation of the tumors.

Reply: We agree that the R1 resection rate was high in this small cohort. We attribute this to selection bias, with likely more TNT patients having locally advanced/aggressive disease (page 11, lines 19-23) All patients with an R1 resection had poorly differentiated, diffuse histology. Please see the added Supplementary Table 1 for detailed information on R1 resection patients. In one case, further esophageal resection was not feasible. In another case, the frozen margin was negative, but pathology review subsequently showed a minute foci of carcinoma.

The survival analyses are not significant in this study. The comparison cannot present neither superiority nor non-inferiority in survival of the two groups. Only a well-designed clinical trial can present either.
Reply: Agree with the comment. We acknowledge this in 3rd paragraph of Discussion and in the Conclusion section.

The 4th paragraph of Introduction should be partially moved to the Methods section.
Reply: We have modified the text as advised.
Changes in the text: Please see that part of the 4th paragraph in Introduction was moved to the 2nd paragraph in Methods.

Reviewer C

This manuscript evaluates perioperative versus total neoadjuvant chemotherapy in gastric cancer. The authors looked at over 100 patients which is impressive, however it is also challenging to gleam true answers from this data as it was insufficiently powered and the majority of the results are not significantly significant. Still this study is very interesting and I understand that it would be difficult to get sufficient N to be privately powered provides us some insight into the question regarding TNT vs chemotherapy. I just caution the authors to use less strong wording with their conclusions. After reviewing, the manuscript requires revisions prior to consideration for publication.

Methods:
1. How did the authors decide to end their data collection period of 2014-2020?
Reply: Data collection stopped in time to allow for analysis/submission to GI ASCO.

2. In the author's discussion they highlight that the manuscript is underpowered. In the study design was a power analysis done? If so, what was the N desired to achieve this power?
Reply: This is a retrospective study, and power analysis was not performed in the study design.

3. Did all patients have a diagnostic laparoscopy at time of diagnosis and staging or were some at a later date just prior to their surgical resection? Please clarify
Reply: All patients had a diagnostic laparoscopy done prior to initiation of chemotherapy, as noted in Methods (page 4, lines 10-11).

4. Please be consistent with using percentages vs patient numbers through the paper. For example: “All patients in the TNT group had baseline PET/CT imaging. Seventeen patients with FDG-avid disease had a repeat PET/CT scan performed at an average of 7.7 weeks from 3 chemotherapy initiation, with 14 out of 17 (82%) having a PET response, defined as >35%”. Please state percentage of the seventeen patients from the whole cohort as you do later with 14/17.

Reply: Added percentage as advised.

Changes in the text: See Page 7, line 15.

5. Please report if this is a significant difference or not: “Median hospital LOS was 7 (IQR: 6-8) and 6 (IQR: 5-8) days in the TNT and perioperative groups”. It seems from the table that the p value was 0.2

Reply: The difference was not significant, included p value in text to clarify.

Changes in the text: Please see Page 8, line 9.

6. “Pathologic complete response (pCR) was defined as the absence of residual invasive cancer 5 1 on hematoxylin and eosin evaluation of the resected specimen” was this reviewed by pathologists or by the researchers?

Reply: pCR was determined by GI cancer subspecialty pathologists.

Results:

1. We agree with the authors that it is important to highlight the reason why physicians chose TNT vs periop and there was likely a majority of reasons. A supplemental table breaking this down would be very interesting to add. As if the reason a patient got TNT was because it was more locally advanced compared to the periop patients that could alter the results. Also if there were unable to tolerate and that is why they did periop as opposed to TNT.

Reply: As noted in the Methods section (2nd paragraph), determination of recommended treatment course was based on interpretation of the treating physician’s clinical documentation. The documented reason for TNT was generally a positive clinical and/or radiographic/PET response to chemotherapy. We can only infer from diagnostic laparoscopy/final pathology results that perhaps the presence of more locally advanced disease may have played a role in decision making (even though not clearly documented after detailed chart review).

2. Re: “A higher proportion of patients in the TNT group received 8 FLOT compared to the perioperative group (79% vs. 31%), consistent with more contemporary 9 interest in TNT based on emerging data in rectal cancer and coincident establishment of FLOT as 10 the new standard perioperative regimen based on the FLOT4 trial”. That is a dramatic difference in chemo used. Could you a breakdown by year or ever two years of the chemo used between the two groups to see if they were similar as time went on?

Reply: Included breakdown of patients treated before and after 2017 to offer general timing of treatment/regimen used (FLOT4 data presented at ASCO 2017).

Changes in the text: See added text to 2nd paragraph of Results section.

3. Please report when median time for when each group of patients were lost to follow up. It is important to understand if there is a difference between these two groups as the number at risk drop at a more steadfast rate in the TNT group.
Reply: Median follow up time was 31 months in the TNT group and 50 months in the periop group. See page 9, line 2.

4. "There was no significant difference in time from last chemotherapy cycle to surgery." Please report the time from last chemo cycle to surgery for each group.
Reply: Please see Table 3 for data, added to text.
Changes in the text: See Page 8, line 8.

Discussion:
1. The majority of patients underwent perioperative chemo compared to TNT. Why is the case? This should be listed a limitation of the study that there is not an equal distribution.
Reply: Most patients received perioperative chemotherapy because this is the standard of care. Added line to address this imbalance.
Changes in the text: See Page 10, lines 16-17.

2. "Indeed, of the 11 TNT patients who had disease recurrence, 3 had linitis plastica, a histology associated with a very poor prognosis due to potential for early metastases and frequent positive surgical margins." What were the stages of the 8 other ones? 3 is not the majority so I am curious of the make up of the rest of the individuals.
Reply: The other patients who had recurrence generally had ypT4/N+ disease, almost all poorly differentiated histology – ypT4N2, ypT3N2, ypT2N3, ypT4N2, ypT4N0, ypT3N3, ypT3N0, ypT4N2.
Changes in the text: See Page 12, lines 1-2.

Conclusion:
1. I appreciate the authors are hedging their results as is appropriate given the sample size and such. As a result I would as such for the following lines "There was no significant difference in RFS 6 or OS between the TNT and perioperative groups. The actual benefit of TNT is unclear and cannot 7 be determined based on this non-randomized, retrospective review." I would ask that the authors further state that this study was not powered for these findings. Further it is hard to determine if there is a the determinant or benefit from TNT from this study. I also understand that it would be very difficult to get the appropriate sample size to have it powered, but it would be appropriate to call it out in the conclusion as well.
Reply: We have modified the text as advised.
Changes in the text: See Page 13, line 3.