nature portfolio

Peer Review File

Longitudinal circulating tumor DNA analysis during treatment of locally advanced resectable gastric or gastroesophageal junction adenocarcinoma: the PLAGAST prospective biomarker study

Corresponding Author: Professor Aziz Zaanan

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
PLAGAST Review
In this original manuscript the authors leverage a modest cohort of 62 patients with GEJ/GC in France collected as part of an observation study (PLAGAST). The cohort findings are all in line with prior findings and offer limited novelty or significance to the field. It is now well accepted that ctDNA kinetics and positivity are strongly prognostic in GC/GEJ and many other cancers. These findings have been shown in gastroesophageal clinical trials (PANDA, ICONIC, EC-CRT-001) as well as multiple retrospective papers (see the following PMID articles among others, 38429311, 31711920, 37728901, 36480779, 32393783, 31988276, 37356061). I would offer the following comments to help aid in the interpretation of the data.

1. In the discussion please comment on the QC failure rate of 15/82 (18.3%) and what samples were used for the assay development (a table for each patient with the sample used (pre-treatment or surgical) and QC status would be informative.

2. Do the patients who fail QC have similar outcomes to those where the assay could be generated? Is there a clinical feature (signet ring cell, diffuse type?) associated with QC failure? This type of information is often glossed over in ctDNA papers and could represent a differentiating feature in this dataset.

3. Please clearly define how frequently and standardized the surveillance was after surgery and/or adjuvant therapy. (the protocol is in French and this reviewer does not speak/read French). This would significantly impact the lead time of 184 days reported and I would strongly consider tempering this section (lines 240-244) and the discussion.

4. It is more standard to group TRG scores as 1-2 vs 3-5. Please consider making this comparison (figure 3D) as opposed to grouping TRG 1/2/3 all together. Are the results the same (see MAGIC analysis from Smyth E, et al., JCO for reference)

5. Lines 185-187: Please revise. It is incorrect to state that ctDNA pre-NAT was associated with inferior RFS (p = 0.068). Please restate to ctDNA at the pre-NAT timepoint was NOT associated with inferior RFS (p = 0.068)

6. To differentiate this from other similar papers perhaps the authors could discuss whether ctDNA should be considered to refine initial staging? In the prognostic groupings after surgery it does seem the ctDNA+ ypT1-3 did worse than the ctDNA- ypT4 patients.

7. In lines 225-226 and figure 4A-B it is hard to understand the denominator for the ctDNA negative patients who recurred. Is this 10/40 (25%)? Please clarify and discuss. Would consider discussing sensitivity here and diver deeper into these 10 patients if possible. Is there something about ctDNA- patients who recur that is different?

8. In the multivariable analyses why was GEJ chosen as the reference? Do the results hold if stomach is used for reference? This could be considered for other categories as well to test robustness of the data.

9. What is the timing of ctDNA during NAT and was this consistent across all patients? I cannot tell from the available data and this is not specified in the methods. What was the median duration of NAT in the cohort? There can be a wide range of neoajuvant time and ctDNA 2 days after starting NAT may be different than 2 months after starting NAT and hard to assess from the provided data.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
This study demonstrates that in patients with LAR G/GEJ adenocarcinoma, “ctDNA positivity during/after NAT and in the postoperative MRD window strongly correlates with inferior outcomes,” and suggests that combining ctDNA with conventional pathological indicators (e.g., TRG, ypTNM) could enable more precise risk stratification. Such findings are highly relevant for optimizing perioperative treatment strategies and exploring future individualized (escalation/de-escalation) therapies. Nevertheless, several issues warrant further consideration and revision:

1. Exclusion of 20 out of 82 patients: The study excluded 20 patients primarily due to sample-quality issues. While some exclusions may be unavoidable, they can introduce bias. At a minimum, the authors should provide details on these excluded patients' clinical and pathological characteristics (e.g., in a Supplementary Table) so readers can assess any differences that might influence the results.

2. Landmark analysis at 12 weeks post-surgery: Employing a 12-week post-surgery “MRD window” and landmark analysis is commendable. However, the authors should specify the causes of early mortality (e.g., disease-related, surgical complications, or unrelated events). Such information is essential for understanding whether, and how, early deaths might skew the interpretation of ctDNA status and outcomes.

3. Potential bias from early deaths in the recurrence cohort: Some patients in the recurrence group died relatively early, possibly before ctDNA could be detected if the tumor burden was minimal. This could bias the ctDNA–RFS relationship by underestimating true recurrence. We recommend highlighting this possibility as a limitation.

4. Seven patients who did not receive NAT: Including seven patients without NAT may introduce bias, given that their treatment differs substantially from standard NAT regimens. For Supplementary Figure 2, we suggest excluding these seven cases or, at minimum, providing a sensitivity analysis without them. The same rationale applies to analyses of ctDNA status in the postoperative MRD phase.

5. Nine patients did not receive ACT: Because ACT can affect both ctDNA levels and clinical outcomes—and the study population received various treatment regimens—we recommend a sensitivity analysis restricted to patients who received ACT. This would help clarify whether treatment heterogeneity confounds the main findings.

6. Value of ctDNA in a multivariate model: The main strength of this study is that it demonstrated that ctDNA correlates with NAT response and long-term prognosis in a multivariate model. However, it is still unclear whether ctDNA itself is superior to multivariate models that integrate established predictive factors (such as TRG and ypTNM), or whether adding ctDNA to these multivariate models significantly improves predictive ability, so this point should be clarified.

7. Detection limits and false-positive/false-negative rates: The authors selected 16 tumor-specific SNVs for mPCR-NGS positivity thresholds, yet provided limited details on how they evaluated or monitored detection limits and potential false-positive/false-negative rates. More extensive discussion would strengthen confidence in the reliability of the ctDNA assay.

8. Cutoff threshold for baseline ctDNA and external validation: Although ROC analysis was used to determine the optimal baseline cutoff (mMTM/mL), an external validation cohort is needed to confirm reproducibility. We recommend acknowledging this requirement as a limitation and encouraging future multicenter or international validation studies.

9. Sampling time points and sample handling: The study states that plasma samples were collected at various stages (before NAT, during NAT, after NAT, and during the postoperative MRD window). However, the authors do not specify how many weeks after starting NAT the blood was drawn or the precise protocol for sample processing and storage—particularly critical for a retrospective ctDNA analysis. They should also clarify why fewer samples were collected during or after NAT.

10. Single-center, relatively small cohort: This is a single-institution study with a modest sample size, limiting its generalizability—particularly for other ethnicities or healthcare settings. We recommend adding a statement in the Discussion on the need for larger, potentially multi-institutional cohorts and international validation to confirm these findings.

(Remarks on code availability)
I checked the code. I did not try to execute the code. The structure of the repository appears to be organized. At least, it clearly shows which code the authors used and what they did with it.

Reviewer #3

(Remarks to the Author)
Dear authors,
I read your work with great interest.

There is a lot of interest in exploring circulating tumor DNA (ctDNA) and its prognostic and predictive capabilities beyond colorectal cancer.

Herein, this work looks at a patient population with still a very high recurrence risk and unmet need to better monitor and stratify patients who may benefit from an alternative approach.

Furthermore, data pertaining to ctDNA dynamics during neoadjuvant therapy is lacking.

The work looks at not only the traditional work of looking at ctDNA and testing during the MRD window and its association with outcomes, but also examines its role and dynamics in the pre-operative setting.

The results provide clinically meaningful benchmarks on the rate of ctDNA positivity at multiple longitudinal clinically important timepoints, as well as quantification also provide insights on patients who were noted to be persistently positive, and hence the worse outcomes.

The hazard ratios and the corresponding differences between recurrence free survival is striking.

This further adds to the growing literature on how to utilize ctDNA best, and incorporation into standard practice beyond colorectal cancer where there is the most literature.

There are of course some limitations in terms of sample size and assay limitations as well as biological aspects that can affect ctDNA detection. But it for sure highlights and signifies as an additional tool/lens to incorporate in addition to standard of care imaging and assessment of pathology and other variables in patients with stomach cancer undergoing neoadjuvant therapy.

I would be highly supportive of publishing this work for publication.

Kudos! The article is written well and tells a nice story. The figures are helpful and add value.

Good Wishes,
Pashtoon.

(Remarks on code availability)
While I am not an expert at reading the code, these analyses are straightforward and should be reproducible.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Within the limitations of this smaller cohort with somewhat heterogenous collection the authors have addressed my comments. This data is in line with existing literature and adds to the overall body of evidence supporting ctDNA as a strongly prognostic feature in gastroesophageal cancers.

(Remarks on code availability)
No concerns.

Reviewer #2

(Remarks to the Author)
The authors have addressed all of the concerns raised in a thorough and satisfactory manner. They have provided appropriate clarifications, conducted relevant sensitivity analyses, and incorporated additional data and figures to support their conclusions. Their responses demonstrate a strong commitment to scientific rigor and transparency, and I believe the manuscript has been significantly strengthened as a result.

I have no further concerns, and I support the revised version of the manuscript in its current form.

(Remarks on code availability)
I checked the code. I did not try to execute the code. The structure of the repository appears to be organized. At least, it clearly shows which code the authors used and what they did with it.

Title: Longitudinal circulating tumor DNA analysis during treatment of locally advanced resectable gastric or gastroesophageal junction adenocarcinoma: the PLAGAST prospective biomarker study

Manuscript ID: NCOMMS-24-76253

Reviewer #1 (Remarks to the Author):

PLAGAST Review

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1. In the discussion please comment on the QC failure rate of 15/82 (18.3%) and what samples were used for the assay development (a table for each patient with the sample used (pre-treatment or surgical) and QC status would be informative.

Response: We thank the reviewer for this comment. The 82 tissue samples used as input for WES generation were a mix of FFPE tissue from primary core biopsy (N=9), resection of primary tumor (N=70), resection of metastasis (N=2), or unknown (N=1). The WES failure rate for core biopsy samples was 33% (3/9), and for resection samples was 15% (11/72). We have added a sentence in the discussion on page 11 to say, "Tumor tissue samples used for WES were from surgical resection or biopsy if surgical tissue was unavailable. The most common reason for WES QC failures in this cohort was insufficient tumor volume."

2. Do the patients who fail QC have similar outcomes to those where the assay could be generated? Is there a clinical feature (signet ring cell, diffuse type?) associated with QC failure? This type of information is often glossed over in ctDNA papers and could represent a differentiating feature in this dataset.

Response: We thank the reviewer for this comment. We generated a table (Supplementary Table 1 in the revised manuscript) comparing the characteristics of 62 patients included in the study vs. the 20 patients excluded due to QC failure. No significant differences were found between any of the clinicopathological factors, recurrence rates, and median follow-up between these 2 subgroups. We have added a statement in the discussion on page 11 to say, "The most common reason for WES QC failures in this cohort was insufficient tumor volume; however, no significant differences were found between any of the clinicopathological factors, recurrence rates, and median follow-up between the subgroup of patients included and those excluded."

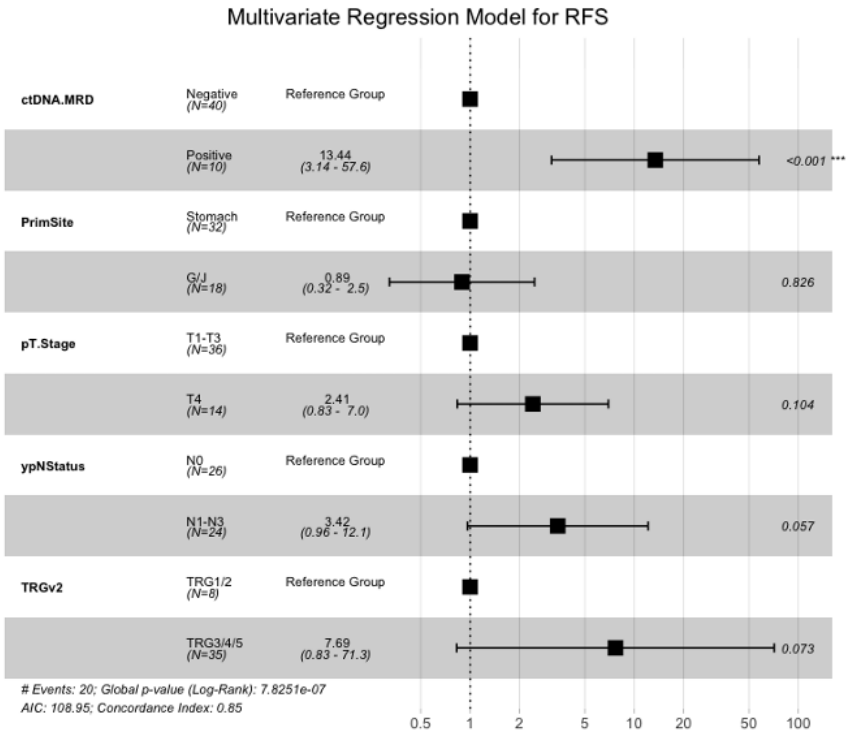
Regarding outcomes: Cox-regression analysis between the two populations did not show any significant differences in the RFS and OS outcomes (Included vs Excluded: RFS: HR = 0.77 (0.35-1.68); P = 0.512, OS: HR = 0.91 (0.36-2.28); P = 0.837). This is important because it shows that the QC-related exclusion of 20 patients in the cohort does not introduce any significant bias in the cohort.

3. Please clearly define how frequently and standardized the surveillance was after surgery and/or adjuvant therapy. (the protocol is in French and this reviewer does not speak/read French). This would significantly impact the lead time of 184 days reported and I would strongly consider tempering this section (lines 240-244) and the discussion.

Response: We thank the reviewer for this comment. Surveillance in the protocol was left to the discretion of the investigator. However, the surveillance proposed in the protocol is in accordance with the French guidelines (https://www.snfge.org/tncd), which recommends CT scan of the thorax, abdomen, and pelvis every 6 months for 3 years following surgical resection, and then once a year for 2 years (Zaanan A et al, Dig Liver Dis. 2018;50(8):768-779). We have added a sentence in the discussion on page 11 to address this comment, "Furthermore, although we report a median lead time of 184 days, we acknowledge that this is specific to the imaging frequency and clinical follow-up as outlined in the protocol."

4. It is more standard to group TRG scores as 1-2 vs 3-5. Please consider making this comparison (figure 3D) as opposed to grouping TRG 1/2/3 all together. Are the results the same (see MAGIC analysis from Smyth E, et al., JCO for reference)

Response: We thank the reviewer for this comment. Indeed, some studies have analyzed the TRG score as 1-2 vs. 3-5 (Smyth EC, JCO 2026;34:2721-2727 / Noble F, Br J Surg 2017;104:1816-1828) and others by grouping TRG score 1-3 vs. 4-5 (Fareed KR, Histopathology 2009;55:399–406 / Fareed KR, BJC 2010;102:1600–1607). In the PLAGAST study, we have considered the TRG 4-5 group as the non-responder group to NAT in order to compare this factor with the negative prognostic impact of ctDNA positivity postoperatively. However, as suggested by the reviewer, we assessed the TRG score by grouping 1-2 vs. 3-5 in the PLAGAST study and we have observed the same results as the comparison of TRG score 1-3 vs. 4-5, i.e., postoperative ctDNA (+) remained the only significant and independent prognostic factor in multivariate analysis for recurrence-free survival (please see figure below). Multivariate analysis for overall survival by pooling TRG score 1-2 vs. 3-5 was not possible given the small number of events and patients in the TRG 1-2 group (N=8). Based on these data, and with your agreement, we proposed retaining the TRG score 1-3 vs. 4-5 comparison.



5. Lines 185-187: Please revise. It is incorrect to state that ctDNA pre-NAT was associated with inferior RFS (p = 0.068). Please restate to ctDNA at the pre-NAT timepoint was NOT associated with inferior RFS (p = 0.068)

Response: We thank the reviewer for this comment and agree. We have modified the statement in the results section on page 6 as below. "At the pre-NAT time point, ctDNA-positive patients showed a trend towards inferior RFS compared to those who tested ctDNA-negative. However, this analysis was not statistically significant (HR= 2.51, 95% CI: 0.94-6.72, p=0.068; median (m) RFS 22.3 months vs. not reached [NR], respectively)."

6. To differentiate this from other similar papers perhaps the authors could discuss whether ctDNA should be considered to refine initial staging? In the prognostic groupings after surgery it does seem the ctDNA+ ypT1-3 did worse than the ctDNA- ypT4 patients.

Response: We thank the reviewer for this comment. To address this comment, we have now added the following sentence to the discussion on page 10, "Our data also showed that postoperative ctDNA status can further refine the prognostic value of pathological tumor staging. As noted above, ypT4 who were MRD-negative had superior 2-year RFS compared to ypT1-3 MRD-positive patients."

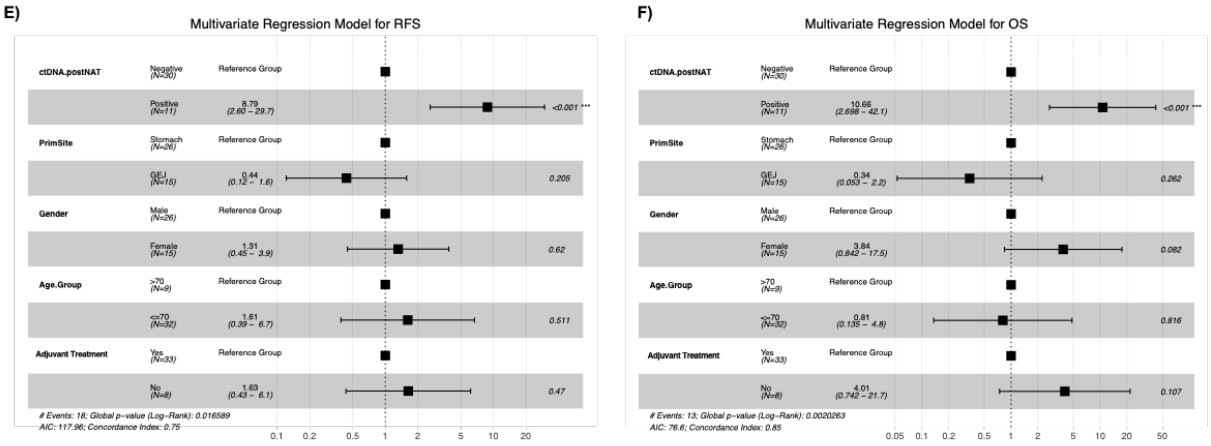
7. In lines 225-226 and figure 4A-B it is hard to understand the denominator for the ctDNA negative patients who recurred. Is this 10/40 (25%)? Please clarify and discuss. Would consider discussing sensitivity here and diver deeper into these 10 patients if possible. Is there something about ctDNA- patients who recur that is different?

Response: We thank the reviewer for this comment. The RFS table shows that the number of events was 35% (14/40). Among these 14 patients, 12 of them recur within 2 years and 10/14 patients recur in the peritoneum, which is considered a low-shedding site. This is consistent with a previous report based on which ctDNA positivity within the MRD window could detect 42% of the recurrences occurring in the peritoneum.

Reference: Nakamura Y, Watanabe J, Akazawa N, et al. ctDNA-based molecular residual disease and survival in resectable colorectal cancer. Nature Medicine. 2024; https://doi.org/10.1038/s41591-024-03254-6.

8. In the multivariable analyses why was GEJ chosen as the reference? Do the results hold if the stomach is used for reference? This could be considered for other categories as well to test robustness of the data.

Response: We thank the reviewer for this comment. Even if we select the stomach as the reference, ctDNA still remains the most significant factor with exactly the same strength of association for both RFS and OS (HR). Overall, it does not change the model, it will only flip the HR for that category. Please see revised Figures 2 E and F below and in the manuscript.



9. What is the timing of ctDNA during NAT and was this consistent across all patients? I cannot tell from the available data and this is not specified in the methods. What was the median duration of NAT in the cohort? There can be a wide range of neoadjuvant time and ctDNA 2 days after starting NAT may be different than 2 months after starting NAT and hard to assess from the provided data.

Response: We thank the reviewer for this comment. We want to clarify that all the annotated "post-neoadjuvant treatment" ctDNA time points are indeed only AFTER the end of NAC. The "on-treatment" time point, as the name suggests, is during neoadjuvant treatment. Some metrics are included below which have also been added to the manuscript on page 6:

Median NAC Duration: 43 days (Q1 – Q3: 42 – 56 days, IQR: 14 days)

Median time from NAC start to ctDNA draw during NAC: 14 days (Q1 – Q3: 14 – 28 days, IQR: 14 days)

Median time from NAC end to ctDNA draw post-NAC: 0 days (Q1 – Q3: 0 – 12 days, IQR: 12 days)

Reviewer #2 (Remarks to the Author):

This study demonstrates that in patients with LAR G/GEJ adenocarcinoma, "ctDNA positivity during/after NAT and in the postoperative MRD window strongly correlates with inferior outcomes," and suggests that combining ctDNA with conventional pathological indicators (e.g., TRG, ypTNM) could enable more precise risk stratification. Such findings are highly relevant for optimizing perioperative treatment strategies and exploring future individualized (escalation/de-escalation) therapies. Nevertheless, several issues warrant further consideration and revision:

1. Exclusion of 20 out of 82 patients: The study excluded 20 patients primarily due to sample-quality issues. While some exclusions may be unavoidable, they can introduce bias. At a minimum, the authors should provide details on these excluded patients' clinical and pathological characteristics (e.g., in a Supplementary Table) so readers can assess any differences that might influence the results.

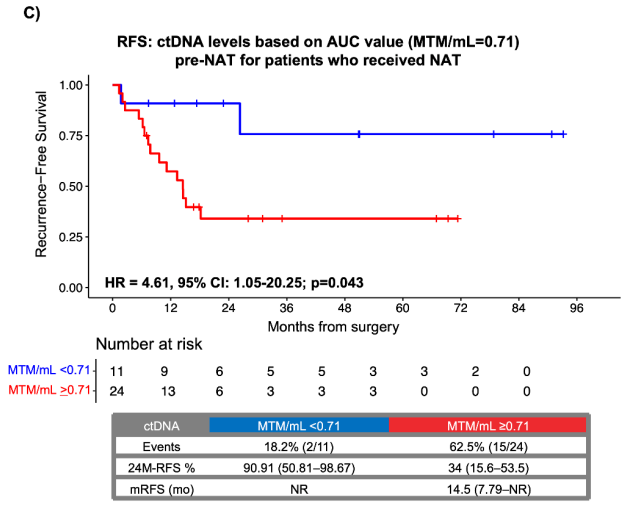
Response: We thank the reviewer for this comment. As mentioned above in response to reviewer 1, we generated a table (Supplementary Table 1) comparing the characteristics of 62 patients included in the study vs. the 20 patients excluded. No significant differences were found between any of the clinicopathological factors, recurrence rates, and median follow-up between these 2 groups.

2. Landmark analysis at 12 weeks post-surgery: Employing a 12-week post-surgery "MRD window" and landmark analysis is commendable. However, the authors should specify the causes of early mortality (e.g.,

disease-related, surgical complications, or unrelated events). Such information is essential for understanding whether, and how, early deaths might skew the interpretation of ctDNA status and outcomes.
Response: We thank the reviewer for this comment. 11 patients with early mortality (event <12 months post-surgery) had disease-related recurrence, with 6/7 ctDNA positive patients to recur within 12 months. 7/11 patients that recurred within 12 months, had a recurrence in the peritoneum, 3 had local/lymph node recurrence, and 1 recurrence in the lung.

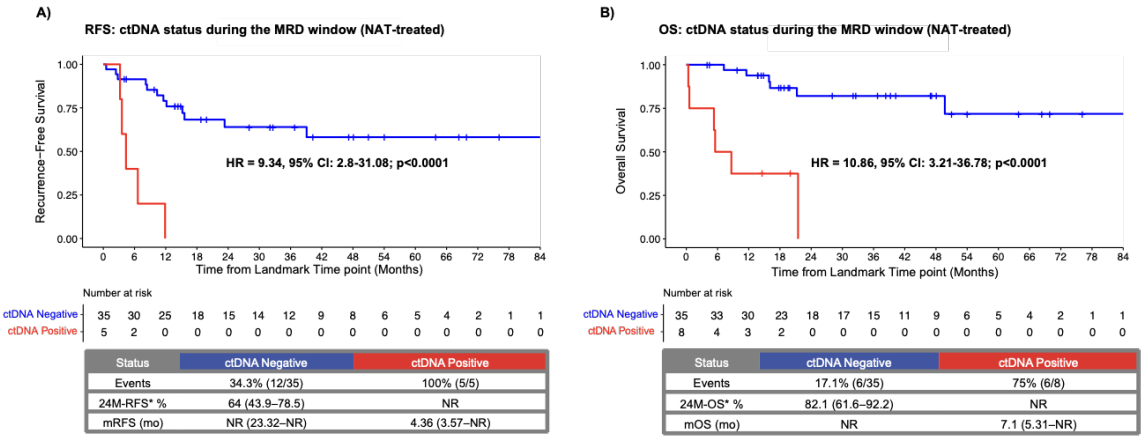
3. Potential bias from early deaths in the recurrence cohort: Some patients in the recurrence group died relatively early, possibly before ctDNA could be detected if the tumor burden was minimal. This could bias the ctDNA–RFS relationship by underestimating true recurrence. We recommend highlighting this possibility as a limitation.
Response: We thank the reviewer for this comment. However, our analysis included a landmark time point to account for immortal time bias. This is applicable to MRD and surveillance. Thus, this limitation has already been addressed in our analysis. For any pre-surgical time points, this is not appropriate since none of the patients progressed prior to surgery. We hope this clarifies the reviewer's question.

4. Seven patients who did not receive NAT: Including seven patients without NAT may introduce bias, given that their treatment differs substantially from standard NAT regimens. For Supplementary Figure 2, we suggest excluding these seven cases or, at a minimum, providing a sensitivity analysis without them. The same rationale applies to analyses of ctDNA status in the postoperative MRD phase.
Response: We thank the reviewer for this comment. Based on the suggestions, we re-did the analysis by excluding no-NAC patients from the analysis and observed similar results, i.e., ctDNA at baseline was not prognostic for RFS and OS, but patients with higher MTM/mL showed worse outcomes (HR = 4.61 (1.05-20.25); P = 0.043). With the new criteria, the threshold shifts to 0.71 MTM/mL. Please see the revised Supplementary Figure 2C below and in the revised manuscript.



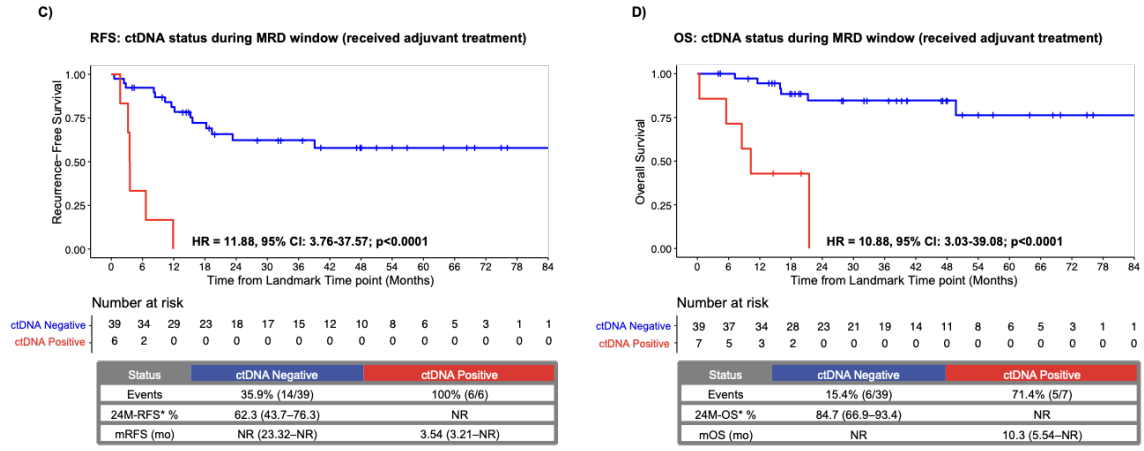
Similarly, for the MRD analysis, we performed a sensitivity analysis by excluding No-NAC patients and observed that ctDNA at MRD was highly prognostic for RFS & OS (RFS: HR = 9.34 (2.8-31.08); P < 0.0001, OS: HR = 10.86 (3.21-36.78); P < 0.0001). Please see revised supplementary figures 3 A and B below and in the revised manuscript.

Supplementary Figure 3: Clinical outcomes by ctDNA status within MRD window for patients who received NAT and adjuvant treatment



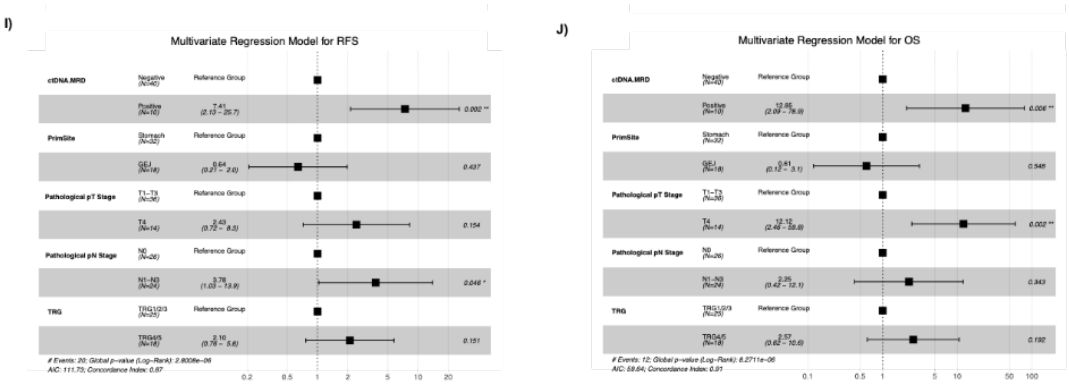
5. Nine patients did not receive ACT: Because ACT can affect both ctDNA levels and clinical outcomes—and the study population received various treatment regimens—we recommend a sensitivity analysis restricted to patients who received ACT. This would help clarify whether treatment heterogeneity confounds the main findings.

Response: We thank the reviewer for this comment. Similarly, we performed a sensitivity analysis by excluding no ACT patients and observed that ctDNA at MRD was highly prognostic for RFS & OS in that subgroup (RFS: HR = 11.88 (3.76–37.57); P < 0.0001, OS: HR = 10.88 (3.03–39.08); P < 0.0001). Please see the revised supplementary figures 3 C and D below and in the revised manuscript.



6. Value of ctDNA in a multivariate model: The main strength of this study is that it demonstrated that ctDNA correlates with NAT response and long-term prognosis in a multivariate model. However, it is still unclear whether ctDNA itself is superior to multivariate models that integrate established predictive factors (such as TRG and ypTNM), or whether adding ctDNA to these multivariate models significantly improves predictive ability, so this point should be clarified.

Response: We thank the reviewer for this comment. In the MRD Multivariate, TRG and ypTNM are already integrated and the results show ctDNA as the most robust prognostic factor. Additionally, we have now selected the stomach as the reference, as per reviewer 1 request. Please see revised Figures 4 I, and J in the manuscript and below.



7. Detection limits and false-positive/false-negative rates: The authors selected 16 tumor-specific SNVs for mPCR-NGS positivity thresholds, yet provided limited details on how they evaluated or monitored detection limits and potential false-positive/false-negative rates. More extensive discussion would strengthen confidence in the reliability of the ctDNA assay.

Response: We thank the reviewer for this comment. Signatera is an analytically and clinically validated assay. Analytical studies have shown over 90% sensitivity at 0.01% VAF, with analytical specificity >99.8%. Clinical validation of Signatera has been published across solid tumors in over 100 publications. Signatera methodology is published in detail in Reinert et al. The variant calling in plasma follows a proprietary algorithm that has QC thresholds for error rates and false positive rates.

Reference: *Reinert T, Henriksen TV, Christensen E, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stage I to III Colorectal Cancer. JAMA Oncology. 2019;5(8):1124-1131.*

8. Cutoff threshold for baseline ctDNA and external validation: Although ROC analysis was used to determine the optimal baseline cutoff (mMTM/mL), an external validation cohort is needed to confirm reproducibility. We recommend acknowledging this requirement as a limitation and encouraging future multicenter or international validation studies.

Response: We thank the reviewer for this comment. We have added a sentence in the discussion on page 11 reflecting the reviewer's suggestion "There is a need for larger, potentially multi-institutional cohorts and international validation studies to confirm the findings of this study."

9. Sampling time points and sample handling: The study states that plasma samples were collected at various stages (before NAT, during NAT, after NAT, and during the postoperative MRD window). However, the authors do not specify how many weeks after starting NAT the blood was drawn or the precise protocol for sample processing and storage—particularly critical for a retrospective ctDNA analysis. They should also clarify why fewer samples were collected during or after NAT.

Response: We thank the reviewer for this comment. Time points were based on the availability of residual tissue and plasma volume. Some more information for the timing of time points relative to NAC are as below which have been added to the manuscript on page 6 and 7:

Median time from NAC start to ctDNA draw during NAC: 14 days (Q1 – Q3: 14 – 28 days, IQR: 14 days)

Median time from NAC end to ctDNA draw post-NAC: 0 days (Q1 – Q3: 0 – 12 days, IQR: 12 days)

Median time from surgery to ctDNA MRD time point: 41 days (Q1 – Q3: 30 – 47 days, IQR: 17 days)

10. Single-center, relatively small cohort: This is a single-institution study with a modest sample size, limiting its generalizability—particularly for other ethnicities or healthcare settings. We recommend adding a statement in the Discussion on the need for larger, potentially multi-institutional cohorts and international validation to confirm these findings.
Response: We thank the reviewer for this comment. As mentioned above, we have added a sentence in the discussion on page 11 reflecting the reviewer's suggestion: "There is a need for larger, potentially multi-institutional cohorts and international validation studies to confirm the findings of this study."

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Reviewer #2 (Remarks on code availability):

I checked the code. I did not try to execute the code. The structure of the repository appears to be organized. At least, it clearly shows which code the authors used and what they did with it.
Response: We thank the reviewer for this comment.

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Furthermore, data pertaining to ctDNA dynamics during neoadjuvant therapy is lacking.

The work looks at not only the traditional work of looking at ctDNA and testing during the MRD window and its association with outcomes, but also examines its role and dynamics in the pre-operative setting.

The results provide clinically meaningful benchmarks on the rate of ctDNA positivity at multiple longitudinal clinically important timepoints, as well as quantification also provide insights on patients who were noted to be persistently positive, and hence the worse outcomes.

The hazard ratios and the corresponding differences between recurrence free survival is striking.

This further adds to the growing literature on how to utilize ctDNA best, and incorporation into standard practice beyond colorectal cancer where there is the most literature.

There are of course some limitations in terms of sample size and assay limitations as well as biological aspects that can affect ctDNA detection. But it for sure highlights and signifies as an additional tool/lens to incorporate in addition to standard of care imaging and assessment of pathology and other variables in patients with stomach cancer undergoing neoadjuvant therapy.

I would be highly supportive of publishing this work for publication.

Kudos! The article is written well and tells a nice story. The figures are helpful and add value.

Good Wishes,
Pashtoon.
Response: We thank the reviewer for this comment.

Reviewer #3 (Remarks on code availability):

While I am not an expert at reading the code, these analyses are straightforward and should be reproducible.
Response: We thank the reviewer for this comment.