

ctDNA clearance and outcomes for resectable lung cancer from NeoCOAST-2

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

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

Summary of the key results

The authors report translational biomarker analyses from the NeoCOAST-2 platform trial evaluating perioperative immunotherapy-based regimens in resectable NSCLC. Using a tumor-agnostic methylation-based ctDNA assay, they demonstrate that early ctDNA clearance during neoadjuvant therapy is strongly associated with pathological response and improved event-free survival. Patients treated with the datopotamab deruxtecan-containing regimen demonstrated the highest rates of ctDNA clearance and pathological complete response, while patients with ctDNA clearance experienced substantially better long-term outcomes than those without clearance. The manuscript further demonstrates that ctDNA clearance and pathological response provide complementary prognostic information, suggesting that blood-based monitoring may identify systemic treatment response beyond assessment of the resected primary tumor.

The principal strengths of the manuscript are the demonstration that early ctDNA clearance during neoadjuvant therapy is strongly associated with event-free survival and complements pathological response. The discordance analyses between ctDNA clearance and pathological response are particularly interesting and suggest that these biomarkers reflect distinct biological aspects of treatment response, with pathology representing local tumor eradication and ctDNA potentially reflecting systemic disease control.

Originality and significance

I believe this work is original and significant. Although ctDNA has emerged as a promising biomarker of molecular residual disease across multiple tumor types, relatively few studies in resectable NSCLC have demonstrated a clear association between early ctDNA clearance during neoadjuvant therapy and longer-term survival outcomes. The manuscript addresses an area of rapidly growing importance as oncology moves toward blood-based monitoring of treatment response and molecular residual disease and is likely to have broad implications extending beyond thoracic oncology. I anticipate this study will be highly cited and serve as a model for similar biomarker analyses in other cancers.

Data & methodology: validity of approach, quality of data, quality of presentation

The overall study design is strong, the ctDNA methodology is appropriate, and the quality of the presentation is excellent. Baseline ctDNA sampling was achieved in nearly the entire study population, providing confidence in the primary analyses. One methodological issue that should be addressed more explicitly is the reduction in evaluable patients at subsequent ctDNA time points. Because the principal conclusions rely on serial ctDNA clearance analyses, it would be helpful for the authors to describe whether patients with missing serial ctDNA samples differed clinically from those included in the analyses and whether any sensitivity analyses were performed to assess the potential impact of missing data.

Appropriate use of statistics and treatment of uncertainties

The statistical analyses are generally appropriate and clearly presented. However, the manuscript would be strengthened by demonstrating more formally whether ctDNA clearance provides prognostic information independent of pathological response and other clinicopathologic variables. The current Kaplan-Meier analyses illustrate the complementary relationship between ctDNA clearance and pathological response but do not establish that ctDNA contributes independent prognostic value. Given the relatively limited number of EFS events, a parsimonious multivariable Cox model including ctDNA clearance, pathological response, stage, and treatment arm (with additional variables if feasible), or comparison of nested prognostic models demonstrating incremental prognostic value, would directly address the manuscript's central hypothesis. In addition, descriptive forest plots demonstrating the association between ctDNA clearance and EFS across clinically relevant subgroups (including treatment arm, PD-L1 status, stage, histology, pathological response, and major pathological response) would be informative without requiring definitive interaction testing. This would substantially help readers understand whether ctDNA clearance is broadly prognostic or whether its association with outcome is driven by particular subgroups. It would be also helpful to see Forest plots for both patients achieving ctDNA clearance and those not achieving clearance.

Conclusions: robustness, validity, reliability

Overall, I believe the conclusions are robust and supported by the data presented. The study convincingly demonstrates that early ctDNA clearance is associated with improved event-free survival and complements pathological response. However, I would encourage the authors to avoid implying that ctDNA-guided treatment adaptation is established by these data. Demonstration of an independent association between ctDNA clearance and outcome beyond pathological response would further strengthen the validity of the central conclusions.

Suggested improvements: experiments, data for possible revision

Several additions could substantially strengthen the manuscript and further elevate its conceptual impact. First, formal analyses demonstrating the independent prognostic value of ctDNA clearance beyond pathological response would strengthen the central message. Second, the manuscript highlights the particularly promising activity of the Dato-DXd-containing regimen, which in the primary NeoCOAST-2 publication appeared to attenuate the dependence of pathological response on PD-L1 expression. Given this observation, it would be informative to determine whether a similar pattern is observed for ctDNA clearance. Specifically, among patients with PD-L1 TPS <1%, were those treated with Dato-DXd more likely to achieve early ctDNA clearance than those receiving the other investigational regimens? Although I recognize that the subgroup sizes are modest, these data appear to be available from the waterfall plots in Extended Data Figure 7 but are not explicitly described or statistically summarized. Including these results—even descriptively—would strengthen the biological interpretation of the study. If Dato-DXd preferentially induces ctDNA clearance in PD-L1-negative tumors, this would support the hypothesis that the regimen may overcome the relative resistance of immunologically "cold" tumors and provide additional mechanistic context for the previously reported pathological response findings. Third, one of the most intriguing findings is the substantially higher ctDNA clearance observed with the datopotamab deruxtecan-containing regimen in the population as a whole. If additional translational data are available, correlative analyses examining systemic immune activation (for example, peripheral immune-cell activation, cytokine responses, T-cell activation, or related biomarkers) could provide important mechanistic insight into why this regimen appears to produce greater molecular responses and would substantially raise the impact of the paper. This is particularly true given the rapidly expanding interest in ADCs. Finally, I believe the manuscript would benefit from additional analysis (or, if not available, discussion) regarding the effect of definitive local therapy on ctDNA clearance. In particular, postoperative ctDNA measurements would help distinguish persistent local disease from true systemic molecular residual disease and would have important implications for future ctDNA-guided treatment adaptation. This issue has important implications for determining whether patients should proceed directly to surgery or whether persistent ctDNA should ultimately prompt escalation of systemic therapy. If postoperative samples were not collected, I encourage the authors to acknowledge this limitation and discuss strategies for determining escalation of systemic therapy versus expediting local therapy.

References: appropriate credit to previous work?

The manuscript appropriately references the existing literature on perioperative immunotherapy, pathological response, and ctDNA biomarkers in resectable NSCLC. The work is well positioned within the context of prior studies, and I have no major concerns regarding attribution of previous work.

Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

The manuscript is exceptionally well written. The abstract accurately reflects the principal findings, the introduction provides appropriate clinical and scientific context, and the discussion appropriately places the work within the evolving field of perioperative biomarker development. Overall, the manuscript is clear, logically organized, and accessible to a broad scientific readership.

Referee #2

(Remarks to the Author)

This manuscript presents updated clinical data from Arms 1, 2, and 4 of the randomized phase 2 NeoCOAST-2 trial (NCT05061550), which evaluated novel perioperative regimens in patients with stage IIA–IIIB NSCLC. This is a clinically

relevant and timely study that addresses an important unmet need in the perioperative treatment of resectable NSCLC. The addition of oleclumab, monalizumab, or datopotamab deruxtecan to standard platinum-based chemotherapy plus durvalumab resulted in encouraging 24-month event-free survival (EFS) rates of 70.5%, 75.7%, and 77.9%, respectively. These findings are particularly relevant given the growing interest in optimizing perioperative chemoimmunotherapy strategies and identifying combinations that may further improve long-term outcomes. The authors also provide updated safety data after completion of adjuvant therapy in all patients. In addition, the exploratory biomarker analyses are of considerable interest, as they suggest the potential utility of a tumor-agnostic ctDNA assay as an early predictor of response.

Major comments:

- Please consider providing survival outcomes according to whether patients received adjuvant therapy and/or whether they completed the planned adjuvant treatment.
- The authors should clarify how ctDNA samples were analyzed: on a sample-by-sample basis or in batches. If batch-based processing was performed, the authors should assess and report whether ctDNA results were comparable across batches.
- It would be informative to provide additional details on the sites of relapse, including the frequency of central nervous system (CNS) recurrences, and to assess whether relapse patterns differed according to treatment arm. I would also be interested to know whether the assay could, at least partially, predict the site of relapse based on methylation patterns or tissue-of-origin signatures. Although this analysis may be highly exploratory, such information could provide additional insight into the potential clinical utility of the assay beyond early response prediction.

Minor comments:

- If available from tumor tissue or sequencing performed elsewhere could it be possible to provide information regarding the genomic alterations. In particular, it would be informative to know whether mutations with known relevance in NSCLC, such as STK11 or KEAP1, were identified, and whether their presence was associated with survival outcomes.
- ctDNA clearance in the absence of pCR was observed in 18/27 patients in Arm 1 (66.7%), 21/30 patients in Arm 2 (70.0%), and 16/29 patients in Arm 4 (55.2%). It would be informative to know how many patients without pCR but with ctDNA clearance experienced favorable survival outcomes. Although this can be inferred from Figure 5, a cancer-specific survival analysis would be useful, as the main objective is to assess the ability of these biomarkers, pCR and ctDNA clearance, to predict cancer-related events.

Referee #3

(Remarks to the Author)

While this work is interesting there are some major concerns

1] The authors state the ADC arm had particularly encouraging activity in the last line of the paper, but all of the CIs are overlapping and the effect of ADC on ctDNA is distinct from immune therapy. I don't think this conclusion has been tested. The conclusion should be that the primary endpoint was hit and published previously and the secondary analysis showed similar EFS results. This maybe due to small numbers.

2] The ctDNA analysis and dynamic changes in NSCLC are not novel. The agnostic approach is probably inferior. If they were to link tissue biology to ctDNA analysis this would be more novel, but as it stands I think both the clinical and ctDNA analysis lack novelty.

Abstract: it should be clear that the primary endpoint has been published previously in Nat. Med. These are exploratory ctDNA analysis.

3] its hard to tell what is new and what was published previously. it is also hard to tell what this adds to the Nat Med paper.

Introduction; The extensive ctDNA data from informed and agnostic analysis such as CM77T should be given in a paragraph - also what is new about this analysis. Issues around agnostic analysis and specificity should be given. Its not clear which part of these analysis are novel. In NSCLC, we know therapy is associated with dynamic changes in ctDNA which correlate with outcome and we know the Dato-DXD arm is more efficacious from the previous publication. We also know the link with pCR.

Results

It is hard to determine what is new and meaningful and what is extended exploratory follow up from the previous publication. I think the authors should focus on the ctDNA analysis. There is nothing new from an efficacy perspective, if anything this clinical data creates more uncertainty. The CIs of the EFS in the 3 analysis are almost identical. I don't think it is possible from this respect to say there is any difference in the arms. I also don't think the speculation that these are 'better results' than in the randomized phase III in the discussion is justified.

Regarding this analysis, it is likely that ADC and immune therapy will have different effects on ctDNA – with more durable remission with immune therapy, and more transient results with ADCs/chemotherapy. This has been shown previously. Therefore all pCRs and ctDNA clearances are not the same.

The authors discuss a number of clinical effects with therapy all of which are exploratory and some of which have a p value of >0.05. The authors should avoid calling out numerical trends and stick to strong association which are predefined. This needs to be addressed in the methods.

The updated safety data could be summarized in 1 paragraph and the rest is supplementary. Did ctDNA positive and

negative patients?

There is no work between the tumor biology and ctDNA status. There is an opportunity to look at TMB/PDL1 and immune RNA signatures – which would be more novel.

The authors state an advantage of the agnostic approach is saving tissue for molecular analysis, which would be novel in lung cancer. But they show no tissue based analysis. This is a major shortcoming.

Please show OS in the main text even if immature.

Discussion

It initially focuses on the efficacy results and suggests cross trial comparison shows the neo-coast regimen are favorable.

This is not statistically correct and should be deleted.

The rest of the discussion is long and does not focus on what is new. Again there is excess detail on potential correlations. I would encourage the authors to focus on what is new.

Methods

There is little detail on the statistical analysis plan and how positive findings were defined in the trial.

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)

I thank the authors for their exceptionally thoughtful and comprehensive responses to the reviewers. The revised manuscript is substantially strengthened. In my view, the authors have satisfactorily addressed my concerns.

In particular, the new multivariable analysis demonstrating that early ctDNA clearance provides prognostic information independent of pCR, stage, and treatment arm substantially strengthens the central conclusion of the manuscript. The subgroup analyses, including the additional analysis according to PD-L1 status, are informative and appropriately presented as exploratory. I also particularly appreciate the addition of the postoperative ctDNA analyses. These data provide important insight into the relationship between systemic molecular response and definitive local therapy and considerably strengthen the biological and clinical interpretation of the findings. All of these additions from this unique clinical trial dataset will be of high interest to the oncology community.

The authors have also appropriately tempered language regarding ctDNA-guided treatment adaptation and the comparisons among treatment arms, while clearly acknowledging the limitations imposed by sample size, immature EFS data, and the absence of additional translational immune analyses.

Overall, I believe the revisions have substantially improved an already strong and clinically important manuscript. I have no additional substantive concerns and support publication of the manuscript in its revised form. I congratulate the authors on an excellent study and an exceptionally thorough response to the review.

Referee #2

(Remarks to the Author)

Thank you very much for the revised version and for the substantial work undertaken in response to the previous comments. The revised manuscript is clearer and has been strengthened by the additional analyses. I have only a few remaining comments, mainly related to clarification and internal consistency of the reported analysis populations and numerical data:

1. The definition of ctDNA clearance and the denominators used for the clearance analyses would benefit from further clarification. In the Results, the authors state that, "Amongst patients who were ctDNA positive at baseline," clearance at C3D1 was observed in 27/53, 30/55, and 29/40 patients in Arms 1, 2, and 4, respectively. However, the Methods indicate that patients classified as non-clearance could have a baseline sample that was ctDNA detected, ctDNA undetected, or unavailable, provided that ctDNA was detected at the on-treatment timepoint. Thus, the clearance-evaluable population does not appear to be restricted exclusively to patients with detectable ctDNA at baseline.
2. There appears to be a minor discrepancy in the distribution of disease stage within the baseline ctDNA-evaluable population. In the Results, ctDNA was detected in 44/61 (72%) patients with stage II disease and 116/134 (87%) patients with stage III disease, corresponding to 195 patients overall. In Supplementary Table 1, however, the baseline ctDNA-evaluable cohorts across the three arms appear to include 59 patients with stage II disease and 136 with stage III disease, also totaling 195 patients. Please verify.
3. In Response recurrence is reported in 6/10 MRD-positive patients and 15/141 MRD-negative patients. However, the subsequent trajectory analysis reports seven recurrences among 107 patients with postoperative clearance and six among eight patients without postoperative clearance. Supplementary Figure 3 also indicates only one recurrence among the additional 36 patients evaluable for postoperative MRD but not included in the trajectory analysis.
4. Response describes EFS and ctDNA endpoints as "pre-specified secondary and exploratory endpoints, respectively," whereas the Methods indicate that both EFS and neoadjuvant ctDNA clearance are secondary endpoints, with analyses of the associations between ctDNA clearance and pCR, MPR, and EFS being exploratory.
5. The exploratory analysis presented in Response 2.2 appears to contain several numerical or formatting errors. For example, the confidence intervals for the 24-month EFS estimates of [] and [] have upper limits of [] and [], which appear to have been inadvertently carried over from the median-EFS column.

6. The recurrence analysis would be clearer if the exact analysis-population definition were used. The rebuttal describes recurrence rates as being calculated "amongst patients who completed surgery," including 7/51 patients in Arm 4, whereas Supplementary Table 4 defines the relevant population as patients in the mITT population who underwent surgical resection and did not have R2 margins. Please align the wording with the population definition used in the analysis and reconcile the denominators with the surgical disposition data in the CONSORT diagram.

Overall, the revised manuscript has addressed the major conceptual issues raised during the previous review and has been substantially improved. The remaining points above appear primarily to involve clarification and harmonization of definitions, denominators, and reported numerical values. Addressing these issues would further improve the transparency and internal consistency of the manuscript.

Referee #3

(Remarks to the Author)

Many thanks for asking me to review this.

The authors state: 'Overall, results from NeoCOAST-2 provide further support for the potential application of ctDNA clearance during neoadjuvant therapy as a biomarker of clinical outcomes.'

I agree, but this isn't the best ctDNA data in lung cancer and doesn't really add anything. ctDNA dynamic are different with different therapy – chemo and ADCs cause bigger initial falls than immune therapy, they seem to miss this which is driving a lot of their findings. They lack the power of a number of previous ctDNA analysis in lung cancer. This is problematic.

The authors also state 'Improved sensitivity of tumour-agnostic ctDNA assays is needed to expand their clinical utility.' Again, I agree, but this article lack the comparisons needed to show this is and improvement.

I dont think this article adds anything to the field.

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Referee 1:

Comment 1.1: The authors demonstrate that early ctDNA clearance during neoadjuvant therapy is strongly associated with pathological response and improved event-free survival. The discordance analyses between ctDNA clearance and pCR are particularly interesting, suggesting these biomarkers reflect distinct biological aspects of treatment response. The work is original and significant. Few studies in resectable NSCLC have demonstrated a clear association between early ctDNA clearance during neoadjuvant therapy and longer-term survival outcomes. The study will likely be highly cited and serve as a model for similar analyses in other cancers.

Response 1.1: We appreciate the reviewer's thoughtful evaluation of our manuscript and acknowledgement of the novelty and potential impact in the perioperative NSCLC setting. We have addressed each of the specific requests raised below.

Comment 1.2: The overall study design is strong, the ctDNA methodology is appropriate, and the quality of the presentation is excellent. Baseline ctDNA sampling was achieved in nearly the entire study population, providing confidence in the primary analyses.

One methodological issue that should be addressed more explicitly is the reduction in evaluable patients at subsequent ctDNA time points. Because the principal conclusions rely on serial ctDNA clearance analyses, it would be helpful for the authors to describe whether patients with missing serial ctDNA samples differed clinically from those included in the analyses and whether any sensitivity analyses were performed to assess the potential impact of missing data.

Response 1.2: We thank the reviewer for highlighting this important methodological consideration. We acknowledge that the reduction in evaluable patients at subsequent ctDNA time points warrants a more detailed description and further characterisation.

Patient demographics and baseline disease characteristics comparing the modified intention-to treat (mITT) and ctDNA-evaluable populations at each time point are presented in **Supplementary Table 1**. We have also added EFS, pCR, and MPR rates to Supplementary Table 1 in the following populations: mITT, ctDNA evaluable at baseline, ctDNA evaluable at neoadjuvant C3D1, and ctDNA evaluable at pre-surgical assessments, to allow for descriptive comparison of clinical outcomes between these populations and to address whether patients with missing serial ctDNA samples differed clinically from those included in the analyses.

Across all arms, the most profound change in number of evaluable patients occurred between the mITT population and the biomarker evaluable population (BEP) with neoadjuvant C3D1 ctDNA clearance-evaluable, while minimal differences were noted between the mITT population and baseline BEP, and between neoadjuvant C3D1 ctDNA-evaluable and pre-surgical ctDNA-evaluable BEPs. In that context, we see below that efficacy outcomes in all arms remain relatively consistent between the mITT population and neoadjuvant C3D1 ctDNA-evaluable BEP, with a minimal numeric improvement in pCR and MPR rates for neoadjuvant C3D1 ctDNA-evaluable BEP, which may be attributed to slightly lower representation of non-pCR/non-MPR patients. This might be expected as the ctDNA-evaluable BEP excludes patients without a neoadjuvant C3D1 sample taken or data available, which may occur as a result of skipped dosing visits or patients withdrawing from the study early. However, altogether, despite missing samples, the similar efficacy outcomes indicate that the neoadjuvant C3D1 ctDNA-evaluable BEP is similar to the mITT population, with the caveat that this is a purely descriptive comparison and our study was not designed to answer this question.

Endpoint	Arm 1 <i>mITT</i>	Arm 1 C3D1 ctDNA evaluable	Arm 2 <i>mITT</i>	Arm 2 C3D1 ctDNA evaluable	Arm 4 <i>mITT</i>	Arm 4 C3D1 ctDNA evaluable
pCR Rate	15/74 (20.3%)	13/53 (24.5%)	18/70 (25.7%)	15/55 (27.3%)	19/54 (35.2%)	16/40 (40.0%)
MPR Rate	31/74 (41.9%)	27/53 (50.9%)	35/70 (50.0%)	30/55 (54.5%)	34/54 (63.0%)	28/40 (70.0%)
EFS 24 mo	70.5% [57.9%, 80.0%]	69.5% [54.5%, 80.4%]	75.7% [63.3%, 84.4%]	77.3% [63.4%, 86.4%]	77.9% [62.4%, 87.6%]	79.6% [61.6%, 89.8%]

We have now explicitly referenced these results at the following locations within the Results section of the revised manuscript:

p7: "Pathological response rates amongst the ctDNA-evaluable populations at baseline, neoadjuvant C3D1, and the pre-surgical assessment were also consistent with the *mITT* population (**Supplementary Table 1**)."

p8: "In the *mITT* population, the 24-month EFS rates (95% CI) were 70.5% (57.9–80.0) in Arm 1, 75.7% (63.4–84.4) in Arm 2, and 77.9% (62.4–87.6) in Arm 4; rates were similar across the ctDNA-evaluable populations at baseline, neoadjuvant C3D1 and the pre-surgical assessment (**Supplementary Table 1**)."

Comment 1.3: The statistical analyses are generally appropriate and clearly presented. However, the manuscript would be strengthened by demonstrating more formally whether ctDNA clearance provides prognostic information independent of pathological response and other clinicopathologic variables. The current Kaplan-Meier analyses illustrate the complementary relationship between ctDNA clearance and pathological response but do not establish that ctDNA contributes independent prognostic value. Given the relatively limited number of EFS events, add a parsimonious multivariable Cox proportional-hazards model for EFS including ctDNA clearance, pathological response, stage, and treatment arm (plus additional covariates if feasible) to formally establish the independent prognostic value of ctDNA clearance beyond pCR. Or compare nested prognostic models (e.g. c-statistic or likelihood-ratio test) to demonstrate incremental prognostic contribution of ctDNA clearance over pCR alone.

Response 1.3: We thank the reviewer for this suggestion. We performed a parsimonious multivariable Cox analysis including baseline disease stage, ctDNA clearance at neoadjuvant C3D1, pCR, and treatment arm. Both ctDNA clearance at neoadjuvant C3D1 and pCR were independently associated with improved EFS: ctDNA clearance reduced event risk by 77.5% (adjusted HR = 0.225; 95% CI = 0.11–0.48; $P < 0.001$) and pCR reduced event risk by 69.7% (adjusted HR = 0.30; 95% CI = 0.11–0.87; $P = 0.026$). Adding ctDNA clearance at neoadjuvant C3D1 to a model with baseline stage, treatment arm and pCR significantly improved model fit ($\Delta\chi^2 = 17.52$; degrees of freedom = 1; $P < 0.0001$), confirming independent prognostic value beyond pCR and other clinical factors.

The manuscript has been revised as follows:

Results (p11): "Using a parsimonious multivariable Cox proportional hazards regression model, both ctDNA clearance at neoadjuvant C3D1 (versus non-clearance; adjusted HR 0.23; 95% CI = 0.11–0.48; $P < 0.001$) and pCR (versus non-pCR; adjusted HR 0.30; 95% CI = 0.11–0.87; $P = 0.026$) emerged as independent prognostic factors for EFS. The addition of ctDNA clearance to a baseline model that included disease stage at baseline, treatment arm and pCR resulted in a statistically significant improvement in model fit ($\Delta\chi^2 = 17.52$; degrees of freedom = 1; $P < 0.0001$)."

Discussion (p17): "ctDNA clearance and pCR were independent prognostic factors in a parsimonious multivariable Cox analysis. These findings are similar to recent reports from CheckMate 77T, which also showed that patients with ctDNA clearance and pCR had the most favourable EFS outcomes, suggesting that ctDNA clearance and pCR may provide complementary prognostic information."

Methods (p43): “To assess the independent prognostic value of ctDNA clearance, we developed a parsimonious multivariable Cox proportional hazards regression model for EFS that included ctDNA clearance at neoadjuvant C3D1, pCR, disease stage at baseline, and treatment arm as covariates. Incremental prognostic value was assessed by likelihood ratio test comparing models with and without ctDNA clearance.”

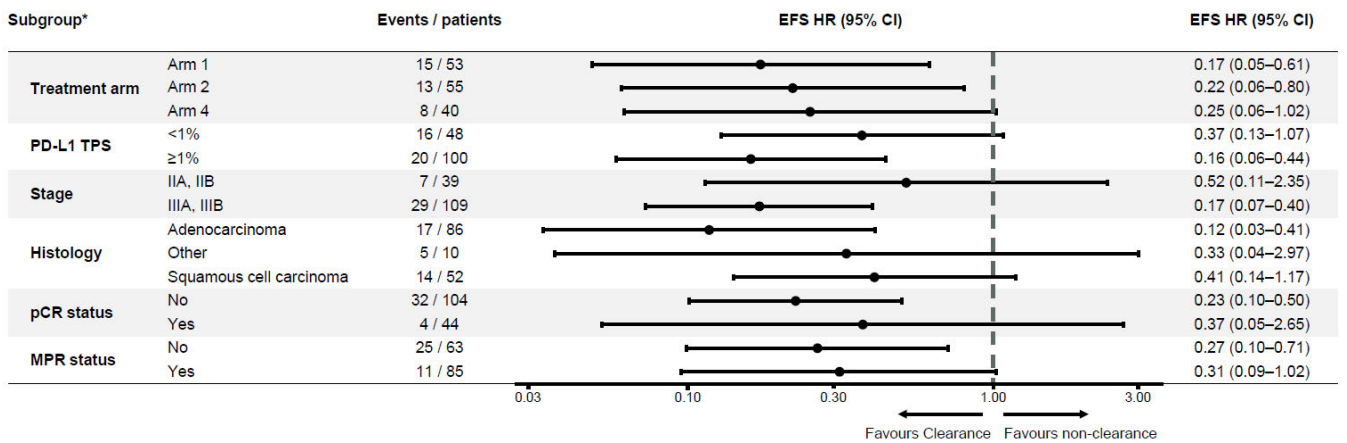
Comment 1.4: Create descriptive forest plots showing the association between ctDNA clearance and EFS across clinically relevant subgroups: treatment arm, PD-L1 status, stage, histology, pathological response, and major pathological response. No formal interaction testing required. This would substantially help readers understand whether ctDNA clearance is broadly prognostic or whether its association with outcome is driven by particular subgroups.

Response 1.4: We thank the reviewer for this recommendation and agree that it will help contextualise whether the prognostic value of ctDNA clearance is consistent across subgroups or concentrated within specific patient populations. We have generated a descriptive forest plot illustrating the association between ctDNA clearance and EFS across clinically relevant subgroups and have incorporated it into the manuscript as follows:

Results (p10):

“Across clinically relevant subgroups defined by histology, PD-L1 TPS status, disease stage, and pathological response, ctDNA clearance at neoadjuvant C3D1 was consistently associated with improved EFS (HR range, 0.12–0.52), although CIs were wide in subgroups with few events (**Supplementary Fig. 2**).”

New Supplementary Figure 2:



Comment 1.5: Show separate forest plots for patients who achieved ctDNA clearance and those who did not achieve clearance.

Response 1.5: We have produced separate forest plots for patients who achieved ctDNA clearance at neoadjuvant C3D1 and those who did not, included here for the reviewer's consideration.

[Redacted text]

We request not to include these forest plots in the revised manuscript, but to present them here in response to this reviewer’s comment for transparency, as these analyses are purely exploratory, and the small subgroup sizes limit statistical power and interpretability.

[Redaction]
Figure Includes
Unpublished Data

[Redaction]
Figure Includes
Unpublished Data

Comment 1.6: The conclusions are robust and supported by the data. The study convincingly demonstrates that early ctDNA clearance is associated with improved EFS and complements pathological response. However, avoid language that implies ctDNA-guided treatment adaptation is established by these data; soften any such claims. Demonstration of an independent association between ctDNA clearance and outcome beyond pathological response would further strengthen the validity of the central conclusions. Formal analyses demonstrating the independent prognostic value of ctDNA clearance beyond pathological response would strengthen the central message.

Response 1.6: We thank the reviewer for their comment. Regarding establishing an independent association between ctDNA clearance and outcome, we have conducted additional formal analyses to evaluate whether ctDNA clearance provides independent prognostic value beyond pathological response based on the reviewer's recommendation, as detailed above.

We have also reviewed the language throughout to ensure that ctDNA-guided treatment adaptation is appropriately framed as a potential avenue rather than an established approach (Discussion p17, 18, 20).

Comment 1.7: The manuscript highlights the particularly promising activity of the Dato-DXd-containing regimen, which in the primary NeoCOAST-2 publication appeared to attenuate the dependence of pathological response on PD-L1 expression. Given this observation, it would be informative to determine whether a similar pattern is observed for ctDNA clearance. Specifically, amongst patients with PD-L1 TPS <1%, were those treated with Dato-DXd more likely to achieve early ctDNA clearance than those receiving the other investigational regimens? Although I recognize that the subgroup sizes are modest, these data appear to be available from the waterfall plots in Extended Data Figure 7 but are not explicitly described or statistically summarised. Including these results—even descriptively would strengthen the biological interpretation of the study. If Dato-DXd preferentially induces ctDNA clearance in PD-L1-negative tumors, this would support the hypothesis that the regimen may overcome the relative resistance of immunologically "cold" tumors and provide additional mechanistic context for the previously reported pathological response findings.

Response 1.7: We agree that further investigation into the ctDNA clearance rates in patients with PD-L1-negative tumours stratified by treatment regimen could provide context to what the biological mechanism underpinning correlation between pathological response and clinical outcomes may be.

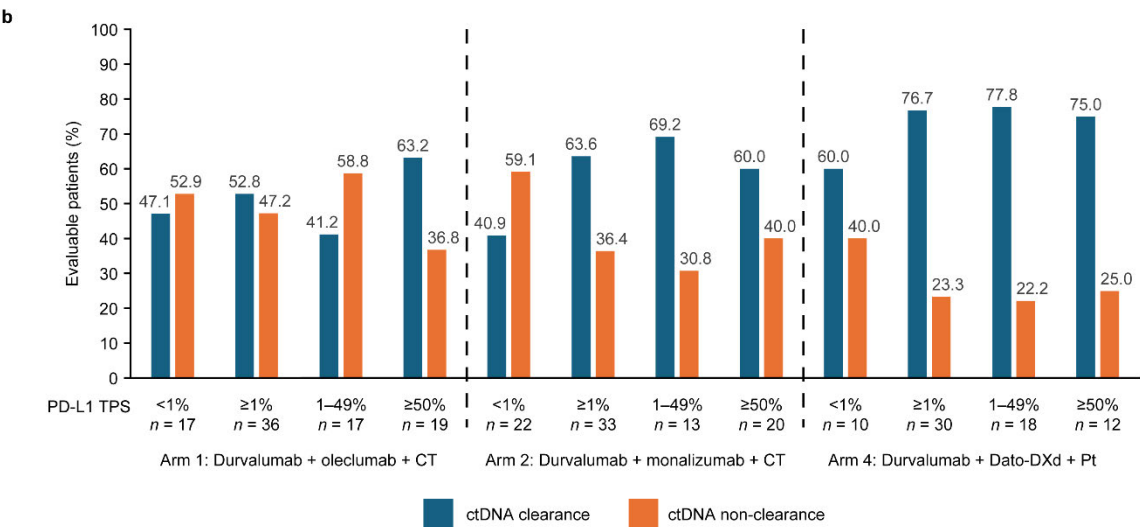
The contribution of PD-L1 TPS status to prognosis and treatment response remains an important consideration in this setting. The PD-L1 negative (i.e. TPS <1%) subgroup of Arm 4 had a numerically higher ctDNA clearance rate and lower non-clearance rate at neoadjuvant C3D1 than the corresponding subgroups of Arm 1 and Arm 2. Though these findings are exploratory and limited by modest subgroup sizes, this pattern is consistent with the numerically higher pCR rate previously observed in Arm 4 compared with Arm 1 and Arm 2 in the PD-L1 negative subgroups. However, ctDNA clearance rates were higher in the PD-L1 positive subgroups across all arms relative to the PD-L1 negative subgroups. Thus, while the data support further investigation of the hypothesis that the Arm 4 regimen may attenuate the dependence of pathologic response on PD-L1 expression relative to other arms, overall, patients across all arms had the best outcomes if they had PD-L1 positive disease. This is further supported by the observation that the 24-month EFS rate favoured patients with PD-L1 positive versus PD-L1 negative disease across all arms.

With the acknowledgement that the analysis is exploratory and is limited in sample size, the data have been descriptively summarised, and a new Supplementary Table and Extended Data figure panel have been generated; this has been included in the revised manuscript as follows:

Results, p10:

“Across all arms, patients with PD-L1 TPS ≥1% had higher ctDNA clearance rates at neoadjuvant C3D1 than patients with PD-L1 TPS <1% (Supplementary Table 5, Extended Data Fig. 5b). Amongst patients with PD-L1 TPS <1%, those in Arm 4 had a numerically higher ctDNA clearance rate (60.0%; 6/10) at neoadjuvant C3D1 than those in Arm 1 (47.1%; 8/17) or Arm 2 (40.9%; 9/22), with a corresponding lower-non-clearance rate (40.0%, 52.9% and 59.1%, respectively).”

New Extended Data Figure 5b



New Supplementary Table 5:

Supplementary Table 5 | ctDNA clearance status at neoadjuvant C3D1 by PD-L1 TPS status across arms.

	PD-L1 TPS				
	<1 %	≥1%	1–49%	≥50%	Total
Arm 1: Durvalumab + oleclumab + CT					
n	17	36	17	19	53
Clearance, n (%)	8 (47.1)	19 (52.8)	7 (41.2)	12 (63.2)	27 (50.9)
Non-clearance, n (%)	9 (52.9)	17 (47.2)	10 (58.8)	7 (36.8)	26 (49.1)
Arm 2: Durvalumab + monalizumab + CT					
n	22	33	13	20	55
Clearance, n (%)	9 (40.9)	21 (63.6)	9 (69.2)	12 (60.0)	30 (54.5)
Non-clearance, n (%)	13 (59.1)	12 (36.4)	4 (30.8)	8 (40.0)	25 (45.5)
Arm 4: Durvalumab + Dato-DXd + Pt					
n	10	30	18	12	40
Clearance, n (%)	6 (60.0)	23 (76.7)	14 (77.8)	9 (75.0)	29 (72.5)
Non-clearance, n (%)	4 (40.0)	7 (23.3)	4 (22.2)	3 (25.0)	11 (27.5)

Comment 1.8: If additional translational data are available, provide correlative analyses examining systemic immune activation (e.g. peripheral immune-cell activation, cytokine responses, T-cell activation) to provide mechanistic insight into greater ctDNA clearance with Dato-DXd.

Response 1.8: Unfortunately, peripheral immune activation data (e.g., immune-cell subsets, cytokine responses, T-cell activation markers) are not available at this time in NeoCOAST-2. We acknowledge that such data would provide valuable mechanistic insight into the greater ctDNA clearance observed with Dato-DXd and will consider this as an important direction for future investigation.

Comment 1.9: Additional analysis (or, if not available, discussion) regarding the effect of definitive local therapy on ctDNA clearance. In particular, postoperative ctDNA measurements would help distinguish persistent local disease from true systemic molecular residual disease and would have important implications for future ctDNA-guided treatment adaptation. This issue has important implications for determining whether patients should proceed directly to surgery or whether persistent ctDNA should ultimately prompt escalation of systemic therapy. If postoperative samples were not collected, I encourage the authors to acknowledge this limitation and discuss strategies for determining escalation of systemic therapy versus expediting local therapy.

Response 1.9: We have performed additional analyses on post-operative ctDNA samples and have included in the manuscript the added prognostic value for EFS that post-operative clearance provides, with improved EFS (HR 0.08) observed in the patients with clearance (molecular residual disease [MRD]-negative) vs non-clearance (MRD-positive) post-surgery.

Further analysis involving EFS outcomes stratified by pre-surgery to adjuvant ctDNA clearance trajectory is provided. Patients who experienced ctDNA clearance before surgery and retained clearance after surgery had the most favourable EFS, whereas those with neither pre-operative nor post-operative ctDNA clearance (persistent non-clearance) had the least favourable outcomes. Notably, patients with pre-operative ctDNA non-clearance who converted to post-operative ctDNA clearance ($n = 30$) had substantially better EFS than those with persistent non-clearance ($n = 2$), which is supportive of the known role of surgery providing curative treatment for many patients. Additionally, a subset of patients ($n = 6$)

who had pre-operative clearance experienced a re-emergence of detected ctDNA (post-operative non-clearance) and had shorter EFS than patients with post-operative clearance with or without pre-operative clearance ($n = 77$ and $n = 30$, respectively), but an improvement compared to those with persistent non-clearance ($n = 2$). These findings indicate that post-operative ctDNA assessment provides clinically relevant prognostic refinement in addition to pre-operative ctDNA status alone when trying to distinguish patients with persistent MRD after definitive local therapy.

Overall, these findings demonstrate the added value of post-operative ctDNA for risk stratification after definitive local therapy and for identifying patients with persistent MRD, although interpretation is limited by small numbers in the post-operative non-clearance groups.

These data have now been added to the manuscript:

Results, p12:

"In post-hoc analyses of post-operative, pre-dose adjuvant C1D1 samples (ctDNA detected: MRD-positive; ctDNA undetected: MRD-negative irrespective of baseline or pre-surgery ctDNA status), across all treatment arms 93.4% (141/151) of patients were MRD-negative, and 6.6% (10/151) were MRD-positive, and MRD-negative status was associated with improved EFS (Cox regression MRD-negative versus MRD-positive at adjuvant C1D1: HR = 0.16; 95% CI = 0.06–0.40). Amongst MRD-positive patients, 60.0% (6/10) had recurrence, compared with 10.6% (15/141) in MRD-negative patients.

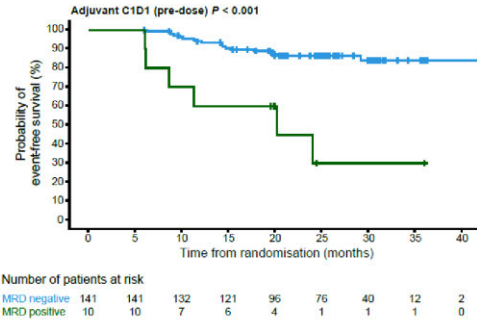
*Moreover, the trajectory of neoadjuvant clearance to post-operative clearance was assessed (evaluable $n = 115$ for both pre-surgery and post-operative clearance). ctDNA clearance following surgery occurred in 107/115 (93.0%) patients including 77 and 30 patients with and without pre-surgical clearance, respectively. Post-surgical clearance was associated with numerically longer EFS versus non-clearance (Cox regression analysis) (HR 0.08; 95% CI = 0.03–0.22). Amongst patients with post-surgical ctDNA clearance, recurrence occurred in 6.5% (7/107) of patients. Of patients without post-surgical ctDNA clearance, recurrence occurred in 100% (2/2) of patients with ctDNA non-clearance at the pre-surgery timepoint and 66.7% (4/6) of those with clearance at the pre-surgery timepoint. Clinical outcomes by ctDNA clearance trajectory from pre-surgery to the post-surgery timepoint are shown in **Supplementary Fig. 3**, though interpretation is limited by small sample size."*

Discussion, p18:

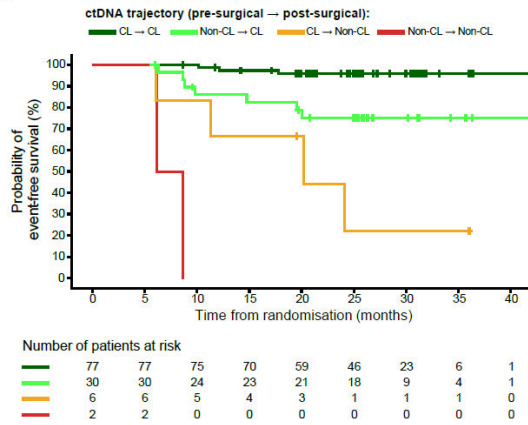
*"While the assessment of MRD via ctDNA detection at a post-surgical landmark was exploratory in NeoCOAST-2, post-hoc analyses of EFS outcomes in a patient population that was ctDNA evaluable at both pre-surgical and post-surgical timepoints demonstrated that ctDNA non-clearance following surgery was limited (eight of 115 evaluable patients, three in Arm 1, four in Arm 2 and one in Arm 4), and that no patients with ctDNA detected following surgery experienced a pCR (**Supplementary Fig. 3**). While these analyses should be interpreted with caution due to small subgroup sample sizes and their exploratory nature, these findings suggest that post-operative ctDNA assessment may provide clinically relevant prognostic refinement alongside pre-operative ctDNA status when trying to distinguish patients with persistent disease and warrant further investigation. This is consistent with CheckMate 77T where, amongst patients who were MRD-negative following surgery and prior to initiation of adjuvant treatment, all who became MRD-positive during adjuvant treatment had disease recurrence, regardless of treatment arm."*

New Supplementary Figure 3:

a



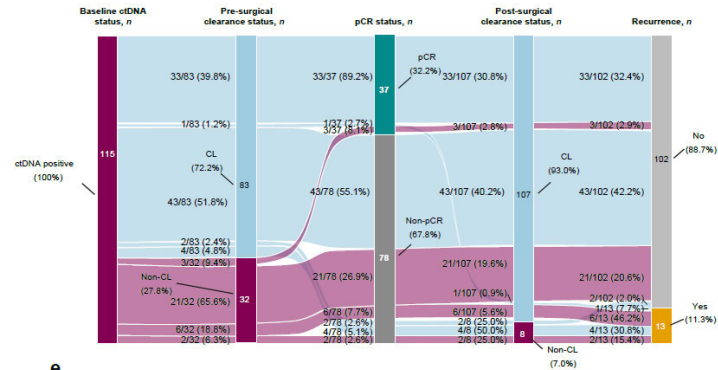
b



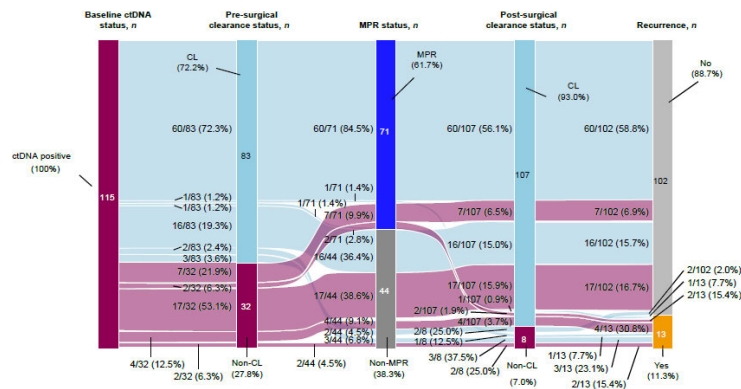
c

ctDNA trajectory (pre-surgical → post-surgical)	Total patients, n (Arm 1/2/4)	Events, n	Median RVT (IQR), %	pCR, n (%)	MPR, n (%)
CL → CL	77 (24 / 25 / 28)	3	2.0 (0.0, 10.0)	34 (44.2)	61 (79.2)
Non-CL → CL	30 (13 / 9 / 8)	7	35.0 (6.2, 55.0)	3 (10.0)	9 (30.0)
CL → Non-CL	6 (2 / 3 / 1)	4	32.5 (18.8, 38.8)	0	1 (16.7)
Non-CL → Non-CL	1 (1 / 1 / 0)	2	45.0 (37.5, 52.5)	0	0

d



e



Comment 1.10: The manuscript appropriately references the existing literature on perioperative immunotherapy, pathological response, and ctDNA biomarkers in resectable NSCLC. No major concerns regarding attribution.

The manuscript is exceptionally well written. The abstract accurately reflects the principal findings, the introduction provides appropriate context, and the discussion appropriately places the work within the evolving field.

Response 1.10: We thank the reviewer for this feedback.

Referee 2

Comment 2.1: This is a clinically relevant and timely study addressing an important unmet need in the perioperative treatment of resectable NSCLC. The 24-month EFS rates across the three arms are encouraging and the exploratory biomarker analyses suggest the potential utility of a tumor-agnostic ctDNA assay as an early predictor of response. Updated safety data after completion of adjuvant therapy in all patients are noted as a strength of the updated report.

Response 2.1: We thank the reviewer for this positive assessment of our work and for recognising the clinical relevance of the NeoCOAST-2 study in the perioperative NSCLC setting. We are pleased that the 24-month EFS outcomes and the translational ctDNA analyses are considered encouraging and informative. We have addressed each of the specific requests raised below.

Comment 2.2: Provide survival outcomes (EFS and other endpoints) stratified by whether patients received adjuvant therapy and/or completed the full planned adjuvant treatment course.

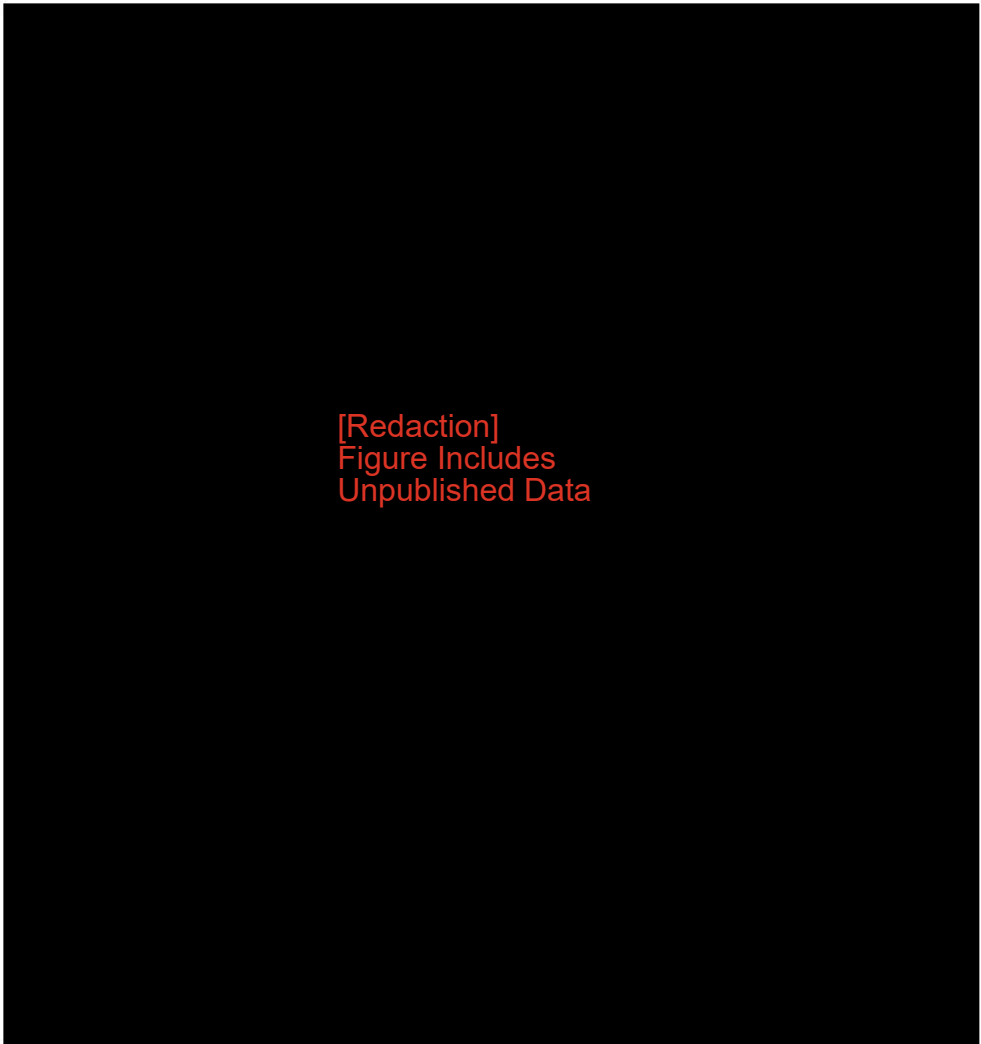
Response 2.2: We have conducted exploratory analyses stratifying outcomes by adjuvant treatment status, pooled across arms given small per-arm subgroup sizes, and present the results in the table and figure below.

Three populations were defined: Population A (no adjuvant, surgery only) includes patients who underwent surgery but did not initiate adjuvant therapy ($n = \blacksquare$); Population B (adjuvant-treated) includes all patients who initiated adjuvant therapy regardless of whether they completed the full planned course ($n = \blacksquare$); and Population C (completed adjuvant) includes only those patients who initiated and completed the full one-year adjuvant treatment course without discontinuation ($n = \blacksquare$).

Interpretation of these findings is limited because, per protocol, patients completing surgery with R0/R1 resection were expected to proceed to adjuvant therapy, and reasons for non-initiation were heterogeneous, including early recurrence/progression, toxicity during neoadjuvant therapy, and patient choice, including some patients declining adjuvant treatment after pCR. Therefore, the surgery only group (Population A) is not directly comparable with the adjuvant-treated populations (B and C). The small size of Population A further limits interpretation.

A limitation of this analysis is that Populations A, B, and C were defined by the post-randomisation treatment pathway patients actually experienced, rather than by baseline randomisation or treatment assignment. All three populations were conditional on undergoing surgery, while initiation of adjuvant therapy in Population B and completion of treatment in Population C additionally required patients to remain alive and event-free for the requisite period. Comparisons of EFS from randomisation are therefore vulnerable to selection related to reaching surgery and, for Populations B and C, to immortal time bias. Population A is also subject to substantial post-randomisation selection and confounding, as non-initiation may reflect early recurrence or progression, disease biology, functional status, treatment-related toxicity, or clinician and patient preference. The EFS and ctDNA clearance estimates should therefore be regarded as descriptive outcomes within clinically selected treatment pathways, rather than as estimates of the causal effect of receiving or completing adjuvant therapy.

Accordingly, these analyses are descriptive and exploratory in nature, and no conclusions can be drawn regarding the contribution of adjuvant therapy to EFS outcomes. We request not to include this analysis in the revised manuscript as the trial was not designed to evaluate the contribution of individual treatment phases and inclusion of these data could invite causal interpretations that the study design does not support.



[Redaction]
Figure Includes
Unpublished Data

Comment 2.3: Clarify whether ctDNA samples were analysed on a sample-by-sample basis or in batches. If batch processing was used, assess and report comparability of ctDNA results across batches.

Response 2.3: We can confirm that ctDNA samples were analysed in batches. Samples were processed across 9 batches, with analyses conducted on an approximately quarterly basis as samples accrued over the course of the study. A detailed description is included below, and we have included an abbreviated version in the Methods section (p41, 42).

This assay was run in a College of American Pathologists (CAP)-accredited laboratory according to written standard operating procedures. All reported results passed individual and batch quality specifications. The platform used for NeoCOAST-2 has undergone repeatability and reproducibility studies that assessed performance across batches that encompass variation in operators, critical reagent lots, instruments, and processing times. For the Repeatability & Reproducibility study that was part of the GRAIL Portrait assay analytical validation, 15 samples from participants with cancer titrated to within 1–4× their in silico LoD95, and 8 samples from participants without cancer were evaluated. At least 18 replicates per sample were tested across three or more batches. Precision was calculated as the percent of total valid replicates per participant with correct cancer/non-cancer classification. Overall precision across all replicates at 1–4× the sample LoD95 was 94.6% (95% CI: 92.3%–96.3%). Median precision across participants at 1–4× the sample LoD95 was 100% (10–90th percentile: 87%–100%).

Additionally, each processing batch includes internal process controls to monitor end-to-end assay performance. The controls are introduced at extraction and processed with the patient samples through sequencing and bioinformatic analysis. The process controls for each batch are evaluated against quality metrics that assess both sequencing coverage recovery and recovery of the targeted cancer-like methylation signal. The acceptance thresholds for these metrics were derived from a statistical model that incorporated observed between-batch and within-batch variation across multiple processing runs, establishing limits at which the assay is performing within its characterised operating range. Furthermore, all batch data is reviewed monthly across multiple (quantitative and qualitative) assay and sequencing quality metrics to assess trends and shifts relative to historical data. Observed performance shifts are investigated and managed per GRAIL's robust quality management system.

Each NeoCOAST-2 batch passing the acceptance thresholds for the quality metrics demonstrates that batch-to-batch variation remained within the bounds characterised during platform development.

The following text was added to the Methods (p41, 42):

"Samples for this analysis were run over nine batches on an approximately quarterly basis. The GRAIL Portrait assay has undergone repeatability and reproducibility studies that assessed performance across batches that encompass variation in operators, critical reagent lots, instruments, and processing times. For the Repeatability & Reproducibility study conducted during analytical validation (Desai M, et al. Cancer Research, 2023), 15 samples from participants with cancer titrated to within 1–4× their in silico LoD95, and eight samples from participants without cancer were evaluated. At least 18 replicates per sample were tested across three or more batches. Precision was calculated as the percent of total valid replicates per participant with correct cancer/non-cancer classification. Overall precision across all replicates at 1–4× the sample LoD95 was 94.6% (95% CI: 92.3%–96.3%). Median precision across participants at 1–4× the sample LoD95 was 100% (10–90th percentile: 87%–100%). Each processing batch included internal process controls to monitor end-to-end assay performance. Each NeoCOAST-2 batch passing the acceptance thresholds for the quality metrics demonstrated that batch-to-batch variation remained within the bounds characterised during platform development."

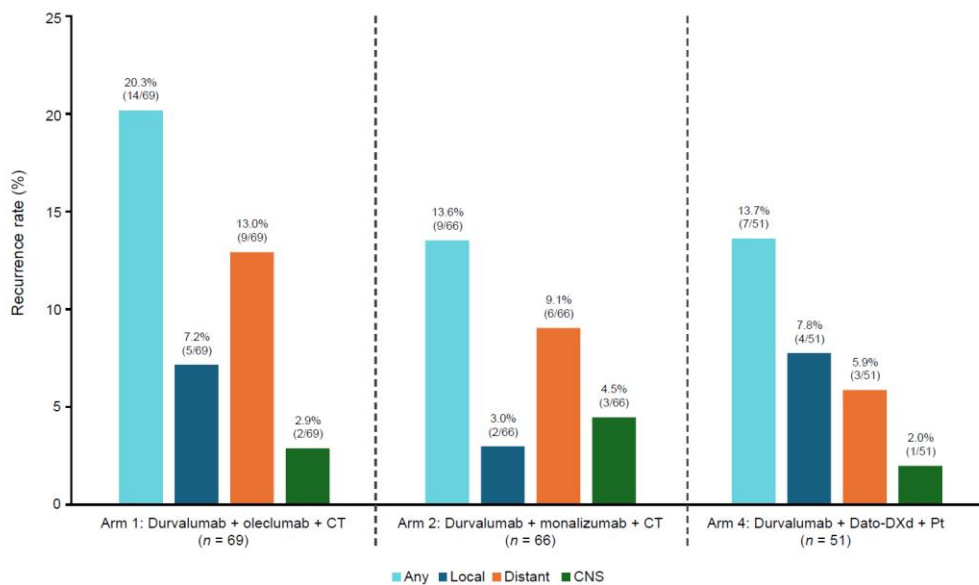
Comment 2.4: Provide details on sites of relapse including frequency of CNS recurrences, and assess whether relapse patterns differed by treatment arm.

Response 2.4: We thank the reviewer for the opportunity to add context to this clinically meaningful question. Details on the site of relapse, including frequency of CNS recurrence, have been added as supplementary material (Supplementary Table 4, Supplementary Figure 1) and in the Results section of the revised manuscript as described below:

Results p8, 9:

*"The rate of disease recurrence amongst patients who completed surgery was 20.3% (14/69) in Arm 1, 13.6% (9/66) in Arm 2, and 13.7% (7/51) in Arm 4 (**Supplementary Table 4**). Local recurrence rates were 7.2% (5/69) in Arm 1, 3.0% (2/66) in Arm 2, and 7.8% (4/51) in Arm 4. Distant recurrence rates were 13.0% (9/69) in Arm 1, 9.1% (6/66) in Arm 2, and 5.9% (3/51) in Arm 4. Brain/central nervous system recurrence occurred in 2.9% (2/69), 4.5% (3/66), and 2.0% (1/51) of patients in Arms 1, 2, and 4, respectively (**Supplementary Fig. 1**)."*

Supplementary Figure 1:



Comment 2.5: Explore whether the methylation-based assay can at least partially predict site of relapse based on methylation patterns or tissue-of-origin signatures. Although this analysis may be highly exploratory, such information could provide additional insight into the potential clinical utility of the assay beyond early response prediction.

Response 2.5: We thank the reviewer for this suggestion. The GRAIL Portrait Assay is a methylation-based platform that classifies ctDNA signal based on a trained machine learning model applied to cfDNA methylation patterns. In principle, methylation-based ctDNA analyses can carry tissue-of-origin information embedded within the methylation signatures of circulating cfDNA fragments. Proof-of-concept for such tissue-of-origin approaches has been demonstrated in the early cancer detection setting, most notably with multi-cancer early detection assays such as GRAIL Galleri (Klein et al., *Ann Oncol* 2021 [https://www.annalsofoncology.org/article/S0923-7534\(21\)02046-9/fulltext/](https://www.annalsofoncology.org/article/S0923-7534(21)02046-9/fulltext/)), which uses methylation patterns to predict cancer site origin. However, for this analysis we used a proprietary NSCLC classifier that does not report tissue-of-origin signals other than NSCLC.

[REDACTED]

Comment 2.6: If available from tumor tissue or sequencing performed elsewhere could it be possible to provide information regarding the genomic alterations in particular, it would be informative to know whether mutations with known relevance in NSCLC, such as *STK11* or *KEAP1*, were identified, and whether their presence was associated with survival outcomes.

Response 2.6: We thank the reviewer for raising the important question of genomic biomarkers. Comprehensive genomic profiling data for *STK11*, *KEAP1*, or other specific somatic mutations are not currently available for the NeoCOAST-2 patient cohort described in this manuscript. The GRAIL Portrait Assay used for ctDNA is methylation-based and somatic alterations within the ctDNA are not assessed. Tumour tissue-based comprehensive genomic profiling via next-generation sequencing panels was not an enrolment requirement; only *EGFR* and *ALK* alteration status was mandated as part of the

inclusion/exclusion criteria and captured in the study database. While additional genomic profiling may be evaluated as part of future exploratory analyses, we acknowledge this as a limitation of the current dataset.

Comment 2.7: ctDNA clearance in the absence of pCR was observed in 18/27 patients in Arm 1 (66.7%), 21/30 patients in Arm 2 (70.0%), and 16/29 patients in Arm 4 (55.2%). It would be informative to know how many patients without pCR but with ctDNA clearance experienced favorable survival outcomes. Although this can be inferred from Figure 5, a cancer-specific survival analysis would be useful, as the main objective is to assess the ability of these biomarkers, pCR and ctDNA clearance, to predict cancer-related events.

Response 2.7: We thank the reviewer for raising this point and we agree this population is of considerable interest. The originally submitted version of the manuscript provided lung cancer-specific events at 24 months amongst patients with ctDNA clearance at neoadjuvant C3D1 (regardless of pCR status) which were very low across all three arms: 2/27 (7.4%), 1/30 (3.3%), and 1/29 (3.4%) in Arms 1, 2, and 4, respectively.

Lung cancer-specific events at 24 months amongst patients with ctDNA clearance at neoadjuvant C3D1 who did not have a pCR were low and generally similar across all three arms: 1/18 (5.5%), 1/21 (4.7%), and 1/16 (6.2%) in Arms 1, 2, and 4, respectively. With the caveat of small sample size, the rate of cancer-specific events at 24 months follows a similar trend to overall EFS in these subgroups showing that patients across all arms with no pCR and no ctDNA clearance at neoadjuvant C3D1 had higher rates of cancer-specific events compared to those with either or both. We have now reported the number of lung cancer-specific EFS events at 24 months for patients with ctDNA clearance but no pCR in the results section:

Results, p11:

“Lung cancer-specific EFS events were similarly uncommon in patients with ctDNA clearance at neoadjuvant C3D1 who did not have a pCR, occurring in 1/18 (5.6%), 1/21 (4.8%), and 1/16 (6.3%) of patients in Arms 1, 2, and 4, respectively.”

	Arm 1	Arm 2	Arm 4	Pooled
no pCR, ctDNA Clearance				
no pCR, no ctDNA Clearance				
pCR, ctDNA Clearance				
pCR, no ctDNA Clearance				

Referee 3

Comment 3.1: The authors state the ADC arm had particularly encouraging activity in the last line of the paper, but all of the CIs are overlapping and the effect of ADC on ctDNA is distinct from immune therapy. I don't think this conclusion has been tested. The conclusion should be that the primary endpoint was hit and published previously and the secondary analysis showed similar EFS results. This may be due to small numbers.

Response 3.1: We agree that formal inter-arm comparisons are not supported; however, we respectively assert that one benefit of a platform study like NeoCOAST-2 is the ability to rapidly signal-find amongst multiple arms to expedite clinical development of promising agents and generate hypotheses for further investigation. To appropriately qualify the interpretation of this observation, the Discussion now identifies the current results as hypothesis-generating and explicitly notes that these findings warrant further investigation (p15). Similarly, the Abstract has been revised to note that findings regarding Arm 4 warrant further investigation. We have also refined and reinforced the previous cautionary language: "*cross-trial and cross-arm comparisons should be interpreted cautiously due to differences in trial design, regimen, analysis methods, heterogenous population characteristics, limited sample sizes, and the non-comparative nature of NeoCOAST-2*" (p14). We have also revised the concluding statement to emphasise the existing point that these findings warrant further investigation (Discussion, p20). Otherwise, the numerically highest 24-month EFS rate in Arm 4 (77.9%) is framed as consistent with the previously reported pCR data from that arm. While the manuscript also acknowledges limited patient numbers and the non-comparative study design as limitations, it is important to consider that, to our knowledge and to date, NeoCOAST-2 remains the only multi-arm global and randomised phase 2 platform trial testing novel regimens on a backbone of standard of care chemoimmunotherapy for patients with operable and resectable NSCLC. This effort represents a major clinical and translational undertaking and despite limitations inherent to the nature of such a study, it is critical to report its exploratory findings to the thoracic oncology field. We feel that with the refinements detailed above, these aspects of the manuscript are fully in line with your comment.

Comment 3.2: The ctDNA analysis and dynamic changes in NSCLC are not novel. The agnostic approach is probably inferior. If they were to link tissue biology to ctDNA analysis this would be more novel, but as it stands I think both the clinical and ctDNA analysis lack novelty. Abstract: it should be clear that the primary endpoint has been published previously in Nat. Med. These are exploratory ctDNA analysis.

Response 3.2: It is unfortunate that this reviewer feels the current report lacks novelty. We respectfully disagree with their statement and appreciate the opportunity to clarify this point in detail. The original manuscript from Cascone *et al.* published in *Nature Medicine* in 2025 reported the primary outcomes of pCR and safety, and secondary endpoints of feasibility of surgery and MPR rates from NeoCOAST-2 Arms 1, 2 and 4. The results of EFS and the ctDNA analyses were not published in that report, and are indeed new secondary endpoints reported here for the first time, representing a substantive contribution beyond the prior publication. In response to this comment, we have strengthened the language in the Abstract (p4), Introduction (p6), and Discussion (p14) to more clearly define what was reported in the previous *Nature Medicine* publication (pCR rate, safety MPR rate, and feasibility of surgery) and what is new in this manuscript (24-month EFS rates, updated safety, and the first association of tumour-agnostic ctDNA clearance with clinical outcomes including EFS).

Regarding the comment raised around the lack of novelty of ctDNA analyses we report in the current work, this referee should note the following points. First, as the Discussion (p20) states: "*To our knowledge, the ctDNA analysis from NeoCOAST-2 is the first to demonstrate the potential utility of a methylation-based tumour-agnostic ctDNA assay for predicting pathological response and EFS with novel agents in the resectable NSCLC setting.*" Prior prospective neoadjuvant NSCLC trials (AEGEAN, CheckMate 816, CheckMate 77T) have all used tumour-informed assays. The present manuscript reports the first application of a tumour-agnostic methylation-based approach in this context, with data on an ADC-containing perioperative regimen – itself a first. Second, the inferiority of tumour-agnostic approaches may be a commonly held

belief, and we acknowledge its technical limitations in our manuscript. However, we do report that ctDNA clearance status with this assay is predictive of pathological outcomes and EFS, and these findings carry novelty. The Discussion states: "*A less sensitive assay may produce false negatives for the presence of ctDNA, thus resulting in lower discriminatory power; despite this, the GRAIL Portrait Assay had sufficient discriminatory power to detect meaningful associations with pCR, MPR, and EFS*" (p19). Third, the manuscript further notes that the tumour-agnostic approach achieved ctDNA evaluability in 98.5% of the mITT population, compared with 24.8–41.0% in tumour-informed trials (CheckMate 816, CheckMate 77T, and AEGEAN), a practically important distinction discussed in the Limitations section (p19).

Comment 3.3: It is hard to tell what is new and what was published previously. It is also hard to tell what this adds to the Nat Med paper.

Response 3.3: The Methods section states that EFS and ctDNA clearance are "additional secondary endpoints" (p39) not covered in the primary report, and the Discussion opens by describing NeoCOAST-2 as "*the first global clinical trial to report integrated clinical and ctDNA data evaluating novel chemoimmunotherapy-based perioperative combination regimens, including an ADC-containing regimen, in the resectable NSCLC setting*" (p14). We have strengthened the language in the Abstract (p4), Introduction (p6), and Discussion (p14) to more clearly define what was reported in the previous *Nature Medicine* 2025 publication (pCR rate, safety, MPR rate, feasibility of surgery) and what results are new in the current manuscript (24-month EFS, updated safety, and the first association of tumour-agnostic ctDNA clearance with clinical outcomes including EFS).

Comment 3.4: The extensive ctDNA data from informed and agnostic analysis such as CM77T should be given in a paragraph - also what is new about this analysis. Issues around agnostic analysis and specificity should be given. It's not clear which part of these analysis are novel. In NSCLC, we know therapy is associated with dynamic changes in ctDNA which correlate with outcome and we know the Dato-DXD arm is more efficacious from the previous publication. We also know the link with pCR.

Response 3.4: The manuscript contains a dedicated discussion of the GRAIL Portrait Assay in the context of prior tumour-informed approaches. The Limitations section provides detailed comparison with CheckMate 816, CheckMate 77T, and AEGEAN – including specific ctDNA evaluability rates (p16, 19); the performance characteristics of the assay (LoD95, classifier specificity of 98%) are included in the Methods (p41, 42); and the Discussion addresses the potential relationship between TMeF and %RVT (p15, 16). The novelty of using a tumour-agnostic methylation-based assay in this setting is stated explicitly, as noted in our response to Comment 3.2.

Regarding specificity and false-positive signals, the Limitations section states: "*The similar outcomes we observed between patients with both ctDNA clearance at neoadjuvant C3D1 and pCR (n = 31) versus patients with pCR alone (n = 13 at neoadjuvant C3D1, which reduced to n = 5 at the pre-surgery timepoint) are likely due to technical limitations of the analysis resulting in variability of ctDNA detection in samples with low TMeF, potentially resulting in false-positive ctDNA detection*" (p19) In the revised manuscript, we have also given a more detailed description of the CheckMate 77T and AEGEAN results in the Discussion (p16, 17) to aid comparison:

"In the CheckMate 77T trial of patients with resectable stage IIA–IIIB NSCLC, amongst patients in the biomarker-evaluable population with detectable ctDNA prior to initiation of neoadjuvant therapy and ctDNA-evaluable at completion of neoadjuvant treatment, HR was 0.41 (95% CI = 0.20–0.86) in patients with ctDNA clearance versus those without for nivolumab and 0.62 (95% CI = 0.31–1.22) for placebo (Cascone, T. et al. Nature, 2026). EFS HRs for nivolumab compared with placebo were 0.48 (95% CI = 0.22–1.02) in patients with ctDNA clearance before surgery and 0.76 (95% CI = 0.40–1.46) in those without. In AEGEAN, patients with pre-surgical ctDNA clearance had improved EFS outcomes versus those without in both the durvalumab arm (HR 0.26; 95% CI = 0.13–0.54) and the placebo arm (HR 0.47; 95% CI = 0.26–0.84). (Reck, M. et al. Ann Oncol, 2024)".

Comment 3.5: It is hard to determine what is new and what is extended exploratory follow-up from the previous publication. The authors should focus on the ctDNA analysis. There is nothing new from an efficacy perspective, if anything this clinical data creates more uncertainty.

Response 3.5: As described in responses to Comments 3.2 and 3.3, we have added explicit signposting in the Abstract (p4), Introduction (p6), and Discussion (p14) distinguishing what is new in this manuscript from the prior publication. Please note, as extensively described in the manuscript, that the EFS data are not extended exploratory follow-up - they are a pre-specified secondary endpoint of the trial reported here for the first time. Similarly, the association between ctDNA clearance, a secondary endpoint in this trial, and EFS is also novel and reported here for the first time.

Given the overlapping CIs and immaturity of the data (maturity of 20.4–27.0% across arms), the EFS results have been framed as hypothesis-generating contextual data rather than as efficacy conclusions in their own right. The manuscript's Limitations already acknowledge "*the limited numbers of patients in individual subgroups, the non-comparative nature of the study and the relative immaturity of the EFS results*" (p20). We have revised the Discussion to ensure the EFS results are clearly positioned as hypothesis-generating and warranting further investigation, rather than as standalone efficacy claims (e.g. Discussion p15, 16, 20).

Comment 3.6: The CIs of the EFS in the 3 analysis are almost identical. I don't think it is possible from this respect to say there is any difference in the arms. I also don't think the speculation that these are 'better results' than in the randomized phase III in the discussion is justified.

Response 3.6: The 24-month EFS rates are 70.5% (95% CI 57.9–80.0), 75.7% (63.4–84.4), and 77.9% (62.4–87.6) in Arms 1, 2, and 4, respectively, with overlapping confidence intervals. Please see our response to comment 3.1 for a description of how we have revised the manuscript to appropriately qualify interpretation of the EFS analyses, which also applies to this comment.

Regarding the cross-trial comparison, we respectfully assert that while definitive conclusions about relative efficacy cannot be drawn, contextualising the EFS data from NeoCOAST-2 alongside findings from other studies in this setting is useful, and deserves to be thoughtfully reported and discussed. To appropriately qualify the interpretation of this observation, the Discussion now identifies the current EFS results as hypothesis-generating (p15), and we have refined the previously included caveat that "*cross-trial and cross-arm comparisons should be interpreted cautiously due to differences in trial design, regimen, analysis methods, heterogenous population characteristics, limited sample sizes, and the non-comparative nature of NeoCOAST-2*" (p14).

We recognise that a numerically higher EFS rate in NeoCOAST-2 does not demonstrate a definitive benefit over the current standard of care. However, the key value of a platform study lies in its signal-finding approach, which identifies promising regimens for further evaluation in confirmatory studies. Therefore, we believe providing standard-of-care context remains relevant, as it helps readers assess whether the observed signals appear sufficiently promising to warrant further investigation.

We have softened our language in the Abstract (p4) and Discussion (p15, 20) to highlight that these results need to be confirmed.

Comment 3.7: Regarding this analysis, it is likely that ADC and immune therapy will have different effects on ctDNA – with more durable remission with immune therapy, and more transient results with ADCs/chemotherapy. This has been shown previously. Therefore, all pCRs and ctDNA clearances are not the same.

Response 3.7: We note that within the NeoCOAST-2 dataset, ctDNA clearance was robustly associated with improved EFS across all three arms pooled (HR 0.22, 95% CI = 0.11–0.45, $P < 0.0001$), and the arm-level HRs were numerically similar (Arm 1: 0.17; Arm 2: 0.22; Arm 4: 0.25). The manuscript does not currently claim equivalence of the biological mechanism underlying ctDNA clearance across arms; however, it is not substantiated that results are more transient with an ADC compared with a novel immunotherapy agent as

the EFS between all three arms, while numerically diverse, have overlapping confidence intervals as we acknowledge above and the trend that ctDNA clearance predicts extended EFS is consistent across arms. We also note that understanding the differential biology of ctDNA dynamics by treatment class is an important priority for potential future work and not the focus of this manuscript.

Discussion, p16:

“Future analysis to understand the differential biology of ctDNA dynamics by treatment class is an important priority and warrants further analysis.”

Comment 3.8: The authors discuss a number of clinical effects with therapy all of which are exploratory and some of which have a p value of >0.05. The authors should avoid calling out numerical trends and stick to strong association which are predefined. This needs to be addressed in the methods.

Response 3.8: We have reviewed the manuscript and removed references to numerical trends in subgroup analyses that did not reach pre-specified statistical significance thresholds. The Statistical Analysis section already describes the analyses as descriptive with Clopper-Pearson confidence intervals, and notes that pooled analyses were performed to achieve sufficient patient numbers. In response to this comment, we have also added explicit statements to the revised manuscript clarifying that: (i) EFS and ctDNA analyses reported here are secondary and exploratory endpoints (Introduction, p6); (ii) no formal multiplicity correction was applied across these endpoints (Methods, p43); and (iii) these analyses should be considered hypothesis-generating only (Discussion, p15). Language in the Results and Discussion describing numerical differences in non-significant subgroup analyses have been qualified.

Comment 3.9: The updated safety data could be summarized in 1 paragraph and the rest is supplementary. Did ctDNA positive and negative patients?

Response 3.9: We thank the Referee for this suggestion. We have further condensed the safety data in the main text.

We have clarified safety in the ctDNA clearance, non-clearance and non-evaluable at neoadjuvant C3D1 subgroups. We have added **Supplementary Table 6** (p94) to the manuscript descriptively summarising neoadjuvant safety outcomes by treatment arm according to ctDNA status at neoadjuvant C3D1, categorised as ctDNA clearance, no ctDNA clearance, and non-evaluable. This table has also been referred to in the manuscript as follows:

Results, p14:

*“Across ctDNA clearance, non-clearance, and non-evaluable subgroups at neoadjuvant C3D1, neoadjuvant safety findings were generally comparable within each treatment arm (**Supplementary Table 6**). Although numerically higher rates of grade ≥3 treatment-emergent AEs or serious AEs were observed amongst patients without versus with ctDNA clearance in Arms 1 and 2, there was no consistent association between ctDNA clearance status and safety outcomes in the neoadjuvant setting. Given the small sample sizes within subgroups, particularly the non-evaluable cohorts, interpretations of these descriptive results are limited.”*

Comment 3.10: There is no work between the tumor biology and ctDNA status. There is an opportunity to look at TMB/PDL1 and immune RNA signatures – which would be more novel. The authors state an advantage of the agnostic approach is saving tissue for molecular analysis, which would be novel in lung cancer. But they show no tissue based analysis. This is a major shortcoming.

Response 3.10: We have performed an exploratory analysis of tumour PD-L1 expression levels in relation to ctDNA clearance rates using data already collected in the trial in response to comment from Referee 1 and have added these results to the manuscript as outlined in the prior response (1.7). RNA immune signatures and TMB are not available in NeoCOAST-2; we have added acknowledgement of this limitation.

Discussion, p20:

“Second, tissue-based molecular analyses beyond PD-L1 and EGFR/ALK sequencing (e.g., tumour mutational burden or RNA immune signatures) are not available, limiting our ability to further describe the relationship between individual tumour biology and ctDNA dynamics.”

Comment 3.11: There is little detail on the statistical analysis plan and how positive findings were defined in the trial.

Response 3.11: The Statistical Analysis section of the manuscript indicates binary endpoints were analysed using number/percentage and 95% Clopper-Pearson CIs; time-to-event endpoints were analysed using the Kaplan-Meier method and Cox proportional hazards regression; RVT-ctDNA correlation was assessed by Spearman's rho; and separate data cut-offs were used for safety/ctDNA (14 October 2025) and EFS (25 March 2026). The sample size rationale references the previously published primary analysis.

In response to this referee's requests, we have:

- Clarified that the EFS and ctDNA endpoints reported in this paper are pre-specified secondary and exploratory endpoints of the trial, respectively (Abstract p4, Introduction p6, Discussion p14, Methods p39)
- Stated that no formal significance threshold or multiplicity adjustment was applied for these secondary endpoints (Methods p43)
- Clarified the non-comparative design of the trial (Discussion p14)
- Noted that results should be interpreted as hypothesis-generating (Discussion p15).

Comment 3.12: Show overall survival (OS) data in the main text, even if immature.

Response 3.12: At the 25 March 2026 data cut-off, the OS data remained immature, with observed OS maturity rates of [REDACTED], [REDACTED], and [REDACTED] for Arms 1, 2, and 4, respectively. We have included a table with the number of OS events per arm below for this peer-review evaluation only. Reporting immature OS data risks being misleading and is unlikely to provide meaningful information at this stage of follow-up. We therefore respectfully request not to include these immature data in the current manuscript, and assure the reviewer that we plan to present these data in a future publication once appropriately mature.

Treatment Arm	Total Patients	OS event	OS Data Maturity (%) as of 25 March 2026
Durvalumab + oleclumab + CT	74	[REDACTED]	[REDACTED]
Durvalumab + monalizumab + CT	70	[REDACTED]	[REDACTED]
Durvalumab + Dato-DXd + Pt	54	[REDACTED]	[REDACTED]

Comment 3.13: Discussion initially focuses on the efficacy results and suggests cross trial comparison shows the neo-coast regimen are favorable. This is not statistically correct and should be deleted. The rest of the discussion is long and does not focus on what is new. Again there is excess detail on potential correlations. I would encourage the authors to focus on what is new.

Response 3.13: While the discussion has been expanded to accommodate the revisions, we have removed duplication and excess text where necessary. As above, we respectfully assert that while definitive conclusions about relative efficacy cannot be drawn, contextualising the EFS data from NeoCOAST-2 alongside findings from other studies in this setting is useful and meaningful. To appropriately qualify the interpretation of this observation, the Discussion now identifies the current EFS and ctDNA results as

hypothesis-generating (p15), in addition to refining the previously included caveat that "*cross-trial and cross-arm comparisons should be interpreted cautiously due to differences in trial design, regimen, analysis methods, heterogenous population characteristics, limited sample sizes, and the non-comparative nature of NeoCOAST-2*" (p14).

Referee 2:

Comment 1: Thank you very much for the revised version and for the substantial work undertaken in response to the previous comments. The revised manuscript is clearer and has been strengthened by the additional analyses. I have only a few remaining comments, mainly related to clarification and internal consistency of the reported analysis populations and numerical data.

Response 1: We appreciate the reviewer's thoughtful evaluation of our manuscript and have addressed each of the specific requests raised below.

Comment 2: The definition of ctDNA clearance and the denominators used for the clearance analyses would benefit from further clarification. In the Results, the authors state that, "Amongst patients who were ctDNA positive at baseline," clearance at C3D1 was observed in 27/53, 30/55, and 29/40 patients in Arms 1, 2, and 4, respectively. However, the Methods indicate that patients classified as non-clearance could have a baseline sample that was ctDNA detected, ctDNA undetected, or unavailable, provided that ctDNA was detected at the on-treatment timepoint. Thus, the clearance-evaluable population does not appear to be restricted exclusively to patients with detectable ctDNA at baseline.

Response 2: We thank the reviewer for highlighting this point. The understanding of the methodology is correct, it is possible to have non-clearance with a detected on-treatment sample and undetected/unevaluable/missing baseline. We agree the sentence in the Results could be improved for clarity and have revised it as follows in the manuscript:

Results (p9): *"Clearance rates for ctDNA at C3D1 of neoadjuvant treatment were 50.9% (n/N = 27/53) in Arm 1, 54.5% (n/N = 30/55) in Arm 2, and 72.5% (n/N = 29/40) in Arm 4 (Fig. 3a)".*

Original sentence: Amongst patients who were ctDNA positive at baseline, the proportion with ctDNA levels below the limit of detection (i.e. clearance) at C3D1 of neoadjuvant treatment was 50.9% (n/N = 27/53) in Arm 1, 54.5% (n/N = 30/55) in Arm 2, and 72.5% (n/N = 29/40) in Arm 4 (Fig. 3a).

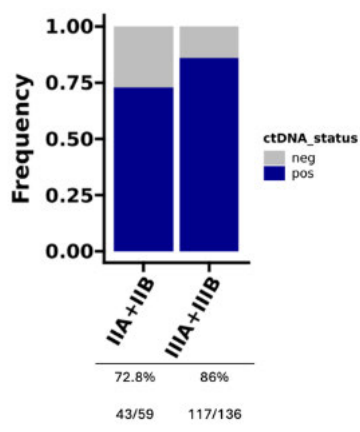
Comment 3: There appears to be a minor discrepancy in the distribution of disease stage within the baseline ctDNA-evaluable population. In the Results, ctDNA was detected in 44/61 (72%) patients with stage II disease and 116/134 (87%) patients with stage III disease, corresponding to 195 patients overall. In Supplementary Table 1, however, the baseline ctDNA-evaluable cohorts across the three arms appear to include 59 patients with stage II disease and 136 with stage III disease, also totaling 195 patients. Please verify.

Response 3: We thank the reviewer for highlighting this inconsistency. We have verified the data, updated Fig. 2b and legend, and reconciled all corresponding text in the manuscript on page 9. The discrepancy reflected the use of an older data cut for the figure, which has now been aligned to the 14Oct2025 data cut, and is consistent with the values presented in **Supplementary Table 1**.

The manuscript has been revised as follows:

Results (p9): "In the overall pooled population, ctDNA was detected less frequently in patients with adenocarcinomas (n/N = 92/125, 74%) versus patients with squamous cell histology (n/N = 57/59, 97%) ($P < 0.0001$), and in patients with stage II (n/N = 43/59, 72.8%) versus stage III disease (n/N = 117/136, 86%) ($P = 0.04$) (Fig. 2b)."

Figure 2b:



Legend: **2b**, Percentage of patients with ctDNA detected at baseline according to tumour histologic type (adenocarcinoma, other, or squamous cell carcinoma) and disease stage (II or III), with data pooled from all treatment arms; ctDNA was detected less frequently in patients with adenocarcinomas versus patients with squamous cell histology (74% versus 97%, Fisher's exact test, $P < 0.0001$) or other tumour histologic types (74% versus 100%, Fisher's exact test, $P = 0.06$), and in patients with stage II (72.8%) versus stage III disease (86%, Fisher's exact test, $P = 0.04$).

Comment 4: In Response recurrence is reported in 6/10 MRD-positive patients and 15/141 MRD-negative patients. However, the subsequent trajectory analysis reports seven recurrences among 107 patients with postoperative clearance and six among eight patients without postoperative clearance. Supplementary Figure 3 also indicates only one recurrence among the additional 36 patients evaluable for postoperative MRD but not included in the trajectory analysis.

Response 4: We thank the reviewer for identifying this. Recurrences for the population subsets who were not included in the MRD analysis and/or ctDNA trajectory analysis has been verified, and the relevant text has been updated and expanded for clarity in the legend for **Supplementary Figure 3**. and have incorporated it into the manuscript as follows:

Supplementary Fig 3 Legend: "...Of the 154 patients, 39/154 were non-evaluable (34/39 missing adjuvant C1D1 sample, and 5/39 not evaluable at adjuvant C1D1 per on-treatment clearance definitions). Of these 39 patients (not depicted), the breakdown with pCR outcomes were as follows: for baseline undetected ($n = 6$ total), six had pre-surgical non-clearance (one pCR, five no pCR). For baseline detected ($n = 33$ total), 17 had pre-surgical non-clearance (one pCR, 16 non-pCR) and 16 had pre-surgical clearance (seven pCR, nine non-pCR). The breakdown with MPR outcomes were as follows: for baseline undetected ($n = 6$ total), six had pre-surgical non-clearance (two MPR, four non-MPR). For baseline detected ($n = 33$ total), 17 had pre-surgical non-clearance (four MPR, 13 non-MPR) and 16 had pre-surgical clearance (11 MPR, five non-MPR). 12 of the 39 unevaluable patients had recurrence; the pathological responses for patients with recurrence were as follows: 0/12 with pCR and 3/12 with MPR. Of those with recurrence and MPR: one with presurgical ctDNA clearance, and two without; of those with non-MPR: one with presurgical clearance, and eight without). An additional 36 patients were evaluable for MRD status (a) but not depicted in panels b–e due to 5/36 patients who were evaluable at the pre-surgical timepoint but not evaluable at adjuvant C1D1, 5/36 patients who were evaluable at adjuvant C1D1 but not at the pre-surgical timepoint, and 26/36 not evaluable for both. Of these 36, 34/36 (94.4%) were MRD-negative and 2/36 (5.6%) were MRD-positive. Recurrence occurred in 8 of these 36 patients; all 8 patients who recurred were MRD-negative and clearance non-evaluable. Pathological responses were as follows: 0/8 with pCR and 2/8 with MPR."

Comment 5: Response describes EFS and ctDNA endpoints as “pre-specified secondary and exploratory endpoints, respectively,” whereas the Methods indicate that both EFS and neoadjuvant ctDNA clearance are secondary endpoints, with analyses of the associations between ctDNA clearance and pCR, MPR, and EFS being exploratory.

Response 5: We thank the reviewer for pointing this out. We agree that the original wording in the response document was not aligned with how the endpoints are described in the Methods. We have revised the text in the response document to clarify that both EFS and neoadjuvant ctDNA clearance were pre-specified secondary endpoints, while the associations between ctDNA dynamics and clinical outcomes was an exploratory endpoint in alignment with the manuscript. This has been updated in the response document as follows:

Response 3.11: “Clarified that the EFS and ctDNA endpoints reported in this paper are *pre-specified secondary endpoints of the trial, and the association between ctDNA dynamics and clinical outcomes is an exploratory endpoint...*”

Comment 6: The exploratory analysis presented in Response 2.2 appears to contain several numerical or formatting errors. For example, the confidence intervals for the 24-month EFS estimates of [REDACTED] and [REDACTED] have upper limits of [REDACTED] and [REDACTED], which appear to have been inadvertently carried over from the median-EFS column.

Response 6: We thank the reviewer for their attention to detail. These numerical discrepancies in formatting have been corrected in the table in the response document.

Comment 7: The recurrence analysis would be clearer if the exact analysis-population definition were used. The rebuttal describes recurrence rates as being calculated “amongst patients who completed surgery,” including 7/51 patients in Arm 4, whereas Supplementary Table 4 defines the relevant population as patients in the mITT population who underwent surgical resection and did not have R2 margins. Please align the wording with the population definition used in the analysis and reconcile the denominators with the surgical disposition data in the CONSORT diagram.

Response 7: We thank the reviewer for their comment. To improve clarity and consistency with the analysis definition, the defined population for **Supplementary Table 4**, **Supplementary Figure 1**, and corresponding text in the manuscript has been revised to specify that recurrence rates were calculated among all patients in the mITT population who underwent surgical resection in alignment with the disposition data presented in the CONSORT diagram. Response 2.4 in the response document has also been updated accordingly. This has been incorporated in the manuscript as follows:

Results (p9): “The rate of disease recurrence *amongst all patients in the mITT who underwent surgery* was 20.3% (14/69) in Arm 1, 13.6% (9/66) in Arm 2, and 13.7% (7/51) in Arm 4 (**Supplementary Table 4**).”

Supplementary Fig 1: Rates of local, distant and CNS recurrence

“Data are presented amongst all patients in the mITT who underwent surgical resection at the data cut-off (25 March 2026).”

Supplementary Table 4 Legend: *“Data are presented per arm and overall amongst all patients in the mITT who underwent surgical resection at the data cut-off (25 March 2026).”*

Comment 8: Overall, the revised manuscript has addressed the major conceptual issues raised during the previous review and has been substantially improved. The remaining points above appear primarily to involve clarification and harmonization of definitions, denominators, and reported numerical values.

Addressing these issues would further improve the transparency and internal consistency of the manuscript.

Response 8: We thank the reviewer for their thoughtful and constructive assessment of our revised manuscript. We appreciate the recognition of the improvements made in response to the previous review. We have carefully addressed the remaining points concerning the clarification and harmonization of definitions, denominators, and reported numerical values, with the aim of further improving the manuscript's transparency and internal consistency.

Referee 1:

Comment 1.1: I thank the authors for their exceptionally thoughtful and comprehensive responses to the reviewers. The revised manuscript is substantially strengthened. In my view, the authors have satisfactorily addressed my concerns.

In particular, the new multivariable analysis demonstrating that early ctDNA clearance provides prognostic information independent of pCR, stage, and treatment arm substantially strengthens the central conclusion of the manuscript. The subgroup analyses, including the additional analysis according to PD-L1 status, are informative and appropriately presented as exploratory. I also particularly appreciate the addition of the postoperative ctDNA analyses. These data provide important insight into the relationship between systemic molecular response and definitive local therapy and considerably strengthen the biological and clinical interpretation of the findings. All of these additions from this unique clinical trial dataset will be of high interest to the oncology community.

The authors have also appropriately tempered language regarding ctDNA-guided treatment adaptation and the comparisons among treatment arms, while clearly acknowledging the limitations imposed by sample size, immature EFS data, and the absence of additional translational immune analyses.

Overall, I believe the revisions have substantially improved an already strong and clinically important manuscript. I have no additional substantive concerns and support publication of the manuscript in its revised form. I congratulate the authors on an excellent study and an exceptionally thorough response to the review.

Response 1.1: We thank the reviewer for their response and their very thorough and thoughtful evaluation of our work; we are very pleased to hear that our responses have satisfied the reviewer's concerns and queries. We sincerely appreciate the time, effort and expertise they devoted to the review process. Their constructive feedback and insightful suggestions have strengthened the rigor and quality of our manuscript and helped us better articulate its contribution to the field.

Referee 2:

Comment 2.1: Thank you very much for the revised version and for the substantial work undertaken in response to the previous comments. The revised manuscript is clearer and has been strengthened by the additional analyses. I have only a few remaining comments, mainly related to clarification and internal consistency of the reported analysis populations and numerical data.

Response 2.1: We appreciate the reviewer's thoughtful evaluation of our manuscript. It is our hope that our responses below and the changes in the resubmitted manuscript have appropriately addressed each of the specific requests raised.

Comment 2.2: The definition of ctDNA clearance and the denominators used for the clearance analyses would benefit from further clarification. In the Results, the authors state that, "Amongst patients who were ctDNA positive at baseline," clearance at C3D1 was observed in 27/53, 30/55, and 29/40 patients in Arms 1, 2, and 4, respectively. However, the Methods indicate that patients classified as non-clearance could have a baseline sample that was ctDNA detected, ctDNA undetected, or unavailable, provided that ctDNA was detected at the on-treatment timepoint. Thus, the clearance-evaluable population does not appear to be restricted exclusively to patients with detectable ctDNA at baseline.

Response 2.2: We thank the reviewer for highlighting this point. The understanding of the methodology is correct, it is possible to have non-clearance with a detected on-treatment sample and undetected/unevaluable/missing baseline. We agree the sentence in the Results could be improved for clarity and have revised it as follows in the resubmitted manuscript:

Results (p9): Revised sentence: "*Clearance rates for ctDNA at C3D1 of neoadjuvant treatment were 50.9% (n/N = 27/53) in Arm 1, 54.5% (n/N = 30/55) in Arm 2, and 72.5% (n/N = 29/40) in Arm 4 (Fig. 3a)*".

Original sentence: *Amongst patients who were ctDNA positive at baseline, the proportion with ctDNA levels below the limit of detection (i.e. clearance) at C3D1 of neoadjuvant treatment was 50.9% (n/N = 27/53) in Arm 1, 54.5% (n/N = 30/55) in Arm 2, and 72.5% (n/N = 29/40) in Arm 4 (Fig. 3a).*

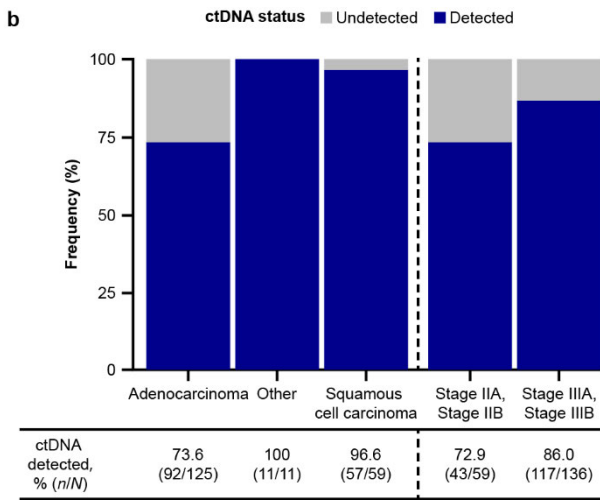
Comment 2.3: There appears to be a minor discrepancy in the distribution of disease stage within the baseline ctDNA-evaluable population. In the Results, ctDNA was detected in 44/61 (72%) patients with stage II disease and 116/134 (87%) patients with stage III disease, corresponding to 195 patients overall. In Supplementary Table 1, however, the baseline ctDNA-evaluable cohorts across the three arms appear to include 59 patients with stage II disease and 136 with stage III disease, also totaling 195 patients. Please verify.

Response 2.3: We thank the reviewer once again for their thoroughness in reviewing our work and for highlighting this inconsistency. We have verified the data, updated **Fig. 2b** and legend, and reconciled all corresponding text in the revised manuscript on page 9. The discrepancy reflected the use of an older data cut for the figure, which has now been aligned to the October 14, 2025 data cut, and is consistent with the values presented in **Supplementary Table 1**.

The manuscript has been revised as follows:

Results (p9): *“In the overall pooled population, ctDNA was detected less frequently in patients with adenocarcinomas (n/N = 92/125, 73.6%) versus patients with squamous cell histology (n/N = 57/59, 96.6%) (P = 0.000087), and in patients with stage II (n/N = 43/59, 72.9%) versus stage III disease (n/N = 117/136, 86.0%) (P = 0.04) (Fig. 2b).”*

Figure 2b:



Legend: 2b, “Percentage of patients with ctDNA detected at baseline according to tumour histologic type (adenocarcinoma, other, or squamous cell carcinoma) and disease stage (II or III), with data pooled from all treatment arms; ctDNA was detected less frequently in patients with adenocarcinomas versus patients with squamous cell histology (73.6% versus 96.6%, two-sided Fisher’s exact test, P = 0.000087) or other tumour histologic types (73.6% versus 100%, two-sided Fisher’s exact test, P = 0.06), and in patients with stage II (72.9%) versus stage III disease (86.0%, two-sided Fisher’s exact test, P = 0.04).”

Comment 2.4: In Response recurrence is reported in 6/10 MRD-positive patients and 15/141 MRD-negative patients. However, the subsequent trajectory analysis reports seven recurrences among 107 patients with postoperative clearance and six among eight patients without postoperative clearance.

Supplementary Figure 3 also indicates only one recurrence among the additional 36 patients evaluable for postoperative MRD but not included in the trajectory analysis.

Response 2.4: We thank the reviewer for identifying this. Recurrences for the population subsets who were not included in the MRD analysis and/or ctDNA trajectory analysis has been verified, and the relevant text has been updated and expanded for clarity in the legend for **Supplementary Figure 3**. and we have incorporated it into the manuscript as follows:

Supplementary Fig 3 Legend: “...Of the 154 patients, 39/154 were non-evaluable (34/39 missing adjuvant C1D1 sample, and 5/39 not evaluable at adjuvant C1D1 per on-treatment clearance definitions). Of these 39 patients (not depicted), the breakdown with pCR outcomes was as follows: for baseline undetected (n = 6 total), six had pre-surgical non-clearance (one pCR, five no pCR). For baseline detected (n = 33 total), 17 had pre-surgical non-clearance (one pCR, 16 non-pCR) and 16 had pre-surgical clearance (seven pCR, nine non-pCR). The breakdown with MPR outcomes were as follows: for baseline undetected (n = 6 total), six had pre-surgical non-clearance (two MPR, four non-MPR). For baseline detected (n = 33 total), 17 had pre-surgical non-clearance (four MPR, 13 non-MPR) and 16 had pre-surgical clearance (11 MPR, five non-MPR). *Twelve of the 39 unevaluable patients had recurrence; the pathological responses for patients with recurrence were as follows: 0/12 with pCR and 3/12 with MPR. Of those with recurrence and MPR: one had presurgical ctDNA clearance, and two did not; of those with non-MPR: one had presurgical clearance, and eight did not.* An additional 36 patients were evaluable for MRD status (a) but not depicted in panels b–e due to 5/36 patients who were evaluable at the pre-surgical timepoint but not evaluable at adjuvant C1D1, 5/36 patients who were evaluable at adjuvant C1D1 but not at the pre-surgical timepoint, and 26/36 not evaluable for both. Of these 36, 34/36 (94.4%) were MRD-negative and 2/36 (5.6%) were MRD-positive. *Recurrence occurred in 8 of these 36 patients; all 8 patients who recurred were MRD-negative and clearance non-evaluable. Pathological responses were as follows: 0/8 with pCR and 2/8 with MPR.*”

Comment 2.5: Response describes EFS and ctDNA endpoints as “pre-specified secondary and exploratory endpoints, respectively,” whereas the Methods indicate that both EFS and neoadjuvant ctDNA clearance are secondary endpoints, with analyses of the associations between ctDNA clearance and pCR, MPR, and EFS being exploratory.

Response 2.5: We agree with the reviewer that the original wording in the response document was not aligned with how the endpoints are described in the Methods. We have revised the text in the response document to clarify that both EFS and neoadjuvant ctDNA clearance were pre-specified secondary endpoints, while the associations between ctDNA dynamics and clinical outcomes was an exploratory endpoint in alignment with the manuscript. This has been updated in the response document as follows:

Revised response 3.11: “Clarified that the EFS and ctDNA endpoints reported in this paper are *pre-specified secondary endpoints of the trial, and the association between ctDNA dynamics and clinical outcomes is an exploratory endpoint...*”

Comment 2.6: The exploratory analysis presented in Response 2.2 appears to contain several numerical or formatting errors. For example, the confidence intervals for the 24-month EFS estimates of [REDACTED] and [REDACTED] have upper limits of [REDACTED] and [REDACTED], which appear to have been inadvertently carried over from the median-EFS column.

Response 2.6: We thank the reviewer for their attention to detail. Upon reviewing the formatting discrepancies noted in our original response, we have re-run the time-to-event analyses for thoroughness; and the updated interpretation, the rectified table and Kaplan-Meier plot have been included below and in the revised response to reviewer 2.2 in the round 1 response document. The rectifications include: (1) updated mEFS estimates and 95% confidence intervals, (2) updated 24-month EFS estimates and 95% confidence intervals, (3) re-generated Kaplan-Meier curves, and (4) the addition of censoring marks on the

KM plots. Importantly, the underlying study population definitions, pCR rates, and ctDNA clearance rates remain unchanged. The following updates have been made in the first response document:

Revised response to reviewer 2.2 comment:

“Three populations were defined: Population A (no adjuvant, surgery only) includes patients who underwent surgery but did not initiate adjuvant therapy (n = [REDACTED]). Population B (adjuvant-treated) includes all patients who initiated adjuvant therapy regardless of whether they completed the full planned course (n = [REDACTED]) and Population C (completed adjuvant) includes only those patients who initiated and completed the full one-year adjuvant treatment course without discontinuation (n = [REDACTED]).

[REDACTED] our overall interpretation is unchanged. Per protocol, patients completing surgery with R0/R1 resection were expected to proceed to adjuvant therapy, and reasons for non-initiation were heterogeneous, including early recurrence/progression, toxicity, and patient choice (including some declining adjuvant treatment after pCR). For example, patients found to have recurrence events at the first post-surgical scan would not have been eligible for adjuvant therapy. The surgery-only group (Population A) is thus not directly comparable with the adjuvant-treated populations (B and C), and the observed differences likely reflect selection bias rather than a treatment effect of adjuvant therapy. The small size of Population A further limits interpretation.”

Accordingly, these analyses remain descriptive and exploratory only, and no conclusions can be drawn regarding the contribution of adjuvant therapy to EFS outcomes. As before, we have elected not to include this analysis in the revised manuscript, as the trial was not designed to evaluate the contribution of individual treatment phases and inclusion of these data could invite causal interpretations that the study design does not support.”

Updated Table:

[Redaction]
Table Includes
Unpublished Data

Updated Figure:

[Redaction]
Figure Includes
Unpublished Data

Comment 2.7: The recurrence analysis would be clearer if the exact analysis-population definition were used. The rebuttal describes recurrence rates as being calculated “amongst patients who completed surgery,” including 7/51 patients in Arm 4, whereas Supplementary Table 4 defines the relevant population as patients in the mITT population who underwent surgical resection and did not have R2 margins. Please align the wording with the population definition used in the analysis and reconcile the denominators with the surgical disposition data in the CONSORT diagram.

Response 2.7: We thank the reviewer for their comment. To improve clarity and consistency with the analysis definition, the defined population for **Supplementary Table 4, Supplementary Figure 1**, and corresponding text in the manuscript has been revised to specify that recurrence rates were calculated among all patients in the mITT population who underwent surgical resection in alignment with the disposition data presented in the CONSORT diagram. Response 2.4 in the response document has also been updated accordingly. This has been incorporated in the manuscript as follows:

Results (p8): “The rate of disease recurrence *amongst all patients in the mITT who underwent surgery* was 20.3% (14/69) in Arm 1, 13.6% (9/66) in Arm 2, and 13.7% (7/51) in Arm 4 (**Supplementary Table 4**).”

Supplementary Fig 1: Rates of local, distant and CNS recurrence

“Data are presented amongst all patients in the mITT who underwent surgical resection at the data cut-off (25 March 2026).”

Supplementary Table 4 Legend: *“Data are presented per arm and overall amongst all patients in the mITT who underwent surgical resection at the data cut-off (25 March 2026).”*

Comment 2.8: Overall, the revised manuscript has addressed the major conceptual issues raised during the previous review and has been substantially improved. The remaining points above appear primarily to involve clarification and harmonization of definitions, denominators, and reported numerical values. Addressing these issues would further improve the transparency and internal consistency of the manuscript.

Response 2.8: We thank the reviewer for their thoughtful and constructive assessment of our revised manuscript. We appreciate the recognition of the improvements made in response to the previous review. We have carefully addressed the remaining points concerning the clarification and harmonization of

definitions, denominators, and reported numerical values, with the aim of further improving the manuscript's transparency and internal consistency.

Referee #3:

Comment 3.1: Many thanks for asking me to review this. The authors state: 'Overall, results from NeoCOAST-2 provide further support for the potential application of ctDNA clearance during neoadjuvant therapy as a biomarker of clinical outcomes.' I agree, but this isn't the best ctDNA data in lung cancer and doesn't really add anything.

Response 3.1: To our knowledge, this is the first report of ctDNA dynamics and their association with outcomes from a global, multi-arm randomized study evaluating novel chemoimmunotherapy-based perioperative combination regimens, including an antibody-drug conjugate (ADC)-containing regimen, and using a tumour-agnostic assay, in the resectable NSCLC setting. Associations between ctDNA clearance, pathological response, and EFS have previously been reported with chemotherapy alone and chemotherapy combined with PD-1 or PD-L1 inhibitors. The current findings extend this evidence by demonstrating that these associations persist across regimens incorporating distinct immunotherapeutic mechanisms, such as those targeting adenosine inhibition through CD73 (oleclumab) or effector cell function through NKG2A (monalizumab), or addition of a TROP2 targeted ADC (Dato-DXd). The consistency of these associations across mechanistically diverse regimens supports the robustness of ctDNA as a biomarker of treatment response and clinical outcomes in this setting. Thus, this study expands the existing evidence by extending the relevance of ctDNA clearance to novel perioperative therapeutic combinations in resectable NSCLC.

Comment 3.2: ctDNA dynamic are different with different therapy – chemo and ADCs cause bigger initial falls than immune therapy, they seem to miss this which is driving a lot of their findings.

They lack the power of a number of previous ctDNA analysis in lung cancer. This is problematic.

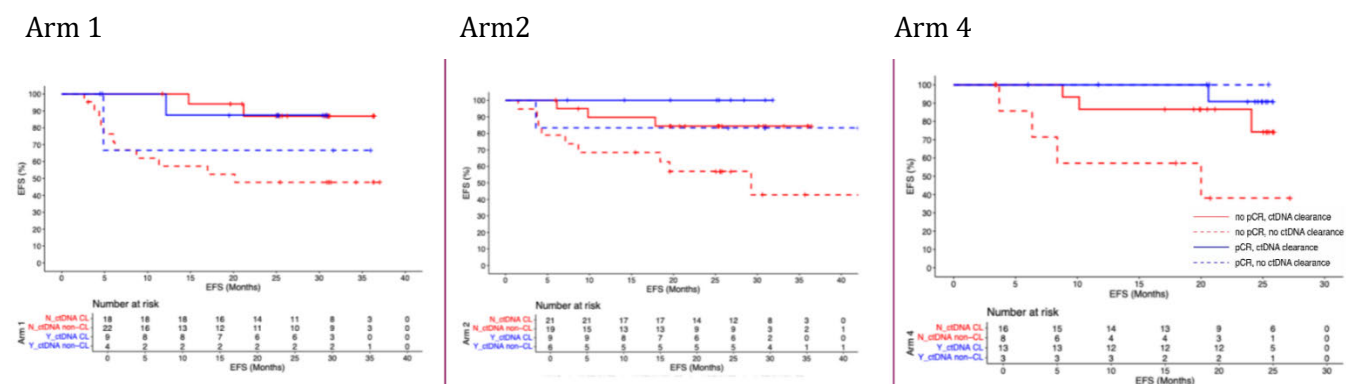
Response 3.2: We believe the reviewer is suggesting that cytotoxics such as chemotherapy or ADCs can induce greater initial reductions in ctDNA than those induced by immunotherapies, but that these reductions are either not sustained or not connected to meaningful clinical outcomes. Specifically, the reviewer may refer to the lower ctDNA clearance observed in the CheckMate 816 trial in the nivolumab + ipilimumab arm (4/36 pts, 11%) versus the chemotherapy (control) arm (10/30 pts, 33%); despite the higher pCR and 24-month EFS rates seen in the nivolumab plus ipilimumab arm (20.4% pCR rate; 60% 24-month EFS rate) compared with the chemotherapy arm (4.6% pCR rate; 48% 24-month EFS rate) (Awad et al, J Clin Oncol 2025 Apr 20;43(12):1453-1462; PMID: 39778121). While this data comes from a limited cohort of patients, we acknowledge that ctDNA clearance frequency and kinetics may vary based on the agents within a treatment regimen, particularly between cytotoxic-containing vs IO-only containing regimens – and we have highlighted in the discussion that “*Understanding differential ctDNA dynamics by treatment class remains an important priority for future work*”. However, all of the arms in NeoCOAST-2 contain both an immunotherapy and a cytotoxic component during the neoadjuvant treatment phase. Also, unlike the observations noted in the nivolumab plus ipilimumab arm versus the chemotherapy arm in CheckMate 816, in our study the ctDNA clearance showed a consistent prognostic association with pCR and EFS outcomes across each arm.

Furthermore, our data is consistent with other regimens that utilize chemotherapy only or combine IO (anti-PD-1/PD-L1 therapies) with cytotoxic therapy. In AEGEAN, both the neoadjuvant chemotherapy plus placebo and chemotherapy plus durvalumab arms demonstrate continued and sustained ctDNA reductions across all cycles of neoadjuvant therapy (C2D1, C3D1, C4D1, and pre-surgery visit) (Reck M et al, Oral Presentation at ESMO 2023 [Abstract LBA59]). In NeoCOAST-2, we observed a similar trend in that ctDNA clearance rates increased in Arms 1, 2 and 4 from C3D1 to pre-surgery visit. In AEGEAN, neoadjuvant ctDNA clearance has been shown to be associated with longer EFS in both the arm with chemotherapy plus placebo and the arm with chemotherapy plus durvalumab. In NeoCOAST-2, we observed a consistent trend of ctDNA clearance association with longer EFS in all arms. If ctDNA dynamics induced by ADC (Dato-DXd)

plus platinum chemotherapy plus durvalumab were inferior to those induced by chemotherapy plus IO, one would hypothesize that Arm 4 would have shorter EFS in ctDNA clearance patients than in Arm 1 or Arm 2, but this was not observed.

To provide further evidence that pooling data across ctDNA clearance vs non-clearance groups across arms provides a meaningful exploratory analysis, we performed the same analysis in each arm separately and have included results of such analysis below. Patients with both ctDNA clearance and pCR had the most favourable EFS outcomes compared with neither in each arm, with similar trends seen across the pCR plus ctDNA clearance, no pCR plus ctDNA clearance, pCR plus ctDNA non-clearance, and no pCR plus ctDNA non-clearance groups. These arm-specific estimates provide supportive evidence of a consistent direction of the association between EFS outcomes and ctDNA clearance and pCR status, and we believe the pooled analysis provides greater precision given the larger denominator, and remains therefore justified.

EFS according to ctDNA clearance status at neoadjuvant C3D1 and pathological response status by Arm



Patients with both ctDNA clearance and pCR had favourable EFS outcomes compared with those with neither. Arm 1: HR (Cox regression) 0.17, 95% CI = 0.02–1.34, $P = 0.09$. Arm 2: No EFS events occurred in patients with both ctDNA clearance and pCR (0/9) vs 9/19 in those with neither (global log-rank $p = 0.021$ across four subgroups; HR not estimable due to zero events. Arm 4: HR (Cox regression) 0.082, 95% CI = 0.0089–0.76, $P = 0.028$).

Comment 3.3: The authors also state ‘Improved sensitivity of tumour-agnostic ctDNA assays is needed to expand their clinical utility.’

Again, I agree, but this article lack the comparisons needed to show this is and improvement.

I don’t think this article adds anything to the field.

Response 3.3: Our intention in the statement cited by the reviewer in their comment 3.3 was to acknowledge that, in general, the sensitivity of tumour-agnostic assays (and even tumour-informed assays), can be improved, rather than to suggest that such improvements are a prerequisite for clinical utility. In our NeoCOAST-2 study, ctDNA non-clearance measured using a tumour-agnostic assay had a robust negative predictive value for non-pCR, and neoadjuvant ctDNA clearance was predictive of prolonged EFS, providing incremental prognostic information beyond pCR. These findings support the prognostic relevance of ctDNA assessment in this setting. Moreover, postoperative ctDNA clearance provided additional prognostic risk stratification after surgery, highlighting the potential value of serial ctDNA assessment in future studies that may evaluate treatment adaptation in high-risk patients. A tumour-informed assay is not a necessity for assessing ctDNA as a biomarker for risk of recurrence in this setting. It is important to highlight that, given the tissue requirements, less than half of patients would be able to have a ctDNA result using a tumour-informed assay, compared with >90% of patients using a tumour-agnostic assay. This means that most patients would not gain prognostic utility from the tumour-

informed assay, despite its greater sensitivity. However, we do believe that further improvements in the sensitivity of tumour-agnostic assays could perhaps identify additional patients with detected ctDNA or non-clearance, who would otherwise be classified as having undetected ctDNA or achieving clearance if using a less sensitive method, and hypothesize that such subset of patients may have a specific risk profile that could be clinically relevant and would warrant further investigation.