nature portfolio

Peer Review File

Neoadjuvant Aumolertinib for Unresectable Stage III EGFR-Mutant Non-Small Cell Lung Cancer

Corresponding Author: Professor Peng Zhang

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this interesting study, Dr. Bian and colleagues reported on a single-arm phase II trial evaluating neoadjuvant aumolertinib for unresectable stage III EGFR-mutant non-small cell lung cancer (NSCLC), alongside a correlative/exploratory analysis using bulk RNA-seq.

From a clinical perspective, the treatment of locally advanced NSCLC patients with targetable mutations remains a critical challenge. This study showed similar results to previous trials, including a respectable response rate, though the pathologic complete response (pCR) or major pathologic response (MPR) rates were not optimal. For patients with stage III disease, the issue is not solely about tumor resectability but also whether resection will offer a survival benefit. Unfortunately, several studies have shown that surgery after neoadjuvant TKI treatment may not provide additional survival benefits. In other words, even if neoadjuvant TKI converts unresectable cases to resectable ones, surgical resection may not lead to improved outcomes. From this standpoint, this study adds more data to the field but does not provide conceptually novel insights. Additionally, the majority of patients with stage III EGFR-mutant NSCLC are not curable. Current guidelines recommend chemoradiotherapy followed by lifelong TKI therapy. The 48-week TKI duration used in this study may not represent the most appropriate timeframe for these patients.

From a translational perspective, the neoadjuvant treatment/surgery approach offers an advantage over chemoradiotherapy because it provides the opportunity to study cancer evolution under treatment. I would like to commend the authors for successfully collecting and analyzing paired pre- and post-treatment specimens, which are invaluable for understanding cancer evolution. However, two main caveats stand out in this study. First, the small sample size is a significant limitation. When analyzing pre-treatment vs. post-treatment, response vs. non-response, and exon 19 vs. exon 21 mutations, the sample size becomes too small to draw definitive conclusions. Even when statistical differences are observed, confounding factors could be at play. Second, while bulk RNA-seq offers valuable insights, it is limited in scope. For such precious samples, spatial transcriptomics may have provided a much more comprehensive analysis.

Reviewer #2

(Remarks to the Author)
Dr Bian et al evaluated treatment and translational outcomes of neoadjuvant aumolertinib in 51 patients with unresectable stage III EGFR-mutant NSCLC. They demonstrated ORR of 70.6%, surgical conversion rate of 45.1%, MPR rate of 21.7%, and pathologic CR rate of 13.0%. In addition, they found increased CD8+ T cells in the post-treatment tissues of responders. Several issues should be clarified.

1. Regarding the endpoint, ORR is the rational endpoint of neoadjuvant therapy in EGFR-mutant NSCLC? This should be clarified. In addition, published ORRs of neoadjuvant EGFR TKIs ranged from 42.0% to 81.0% (mostly around 50% including neoadjuvant osimertinib, 52%; PMID: 39028931). Authors should discuss and compare other ORRs, MPR, pathologic CR, and surgical conversion rates in prospective studies.
2. Although PACIFIC trial included EGFR-mutant NSCLC cases, guideline did not support the use of consolidation checkpoint blockade after concurrent chemoradiotherapy. This should be revised in the introduction.
3. Regarding whole-transcriptome data, authors should evaluate the TCGA immune signature (PMID: 29628290). In

addition, the comparison of pre-treatment and post-treatment transcriptome should be explored using Shankey diagram and GOplot correlation between tumors and TME.
4. Authors should clarified unknown EGFR mutation in the Table 1. Authors can evaluate EGFR mutation subtype using RNA-seqeuncing data.
5. Do authors evaluate the ctDNA dynamics of pre- and post-treatment settings? If possible, please provide the ctDNA data for this population.
6. Therapy-induced evolution of oncogene-driven lung cancer is very important. In surgical samples, cell states may be "residual disease" based on the published data (PMID: 32822576). In addition, drug-persister state may exist in post-surgical samples regardless of response states. Please analyze these.

Reviewer #3

(Remarks to the Author)
This trial explored the efficacy and safety of neoadjuvant Aumolertinib for unresectable stage III EGFR-mutant NSCLC. It revealed that 23 subjects proceeded to surgery among the 51 patients enrolled, with an acceptable safety profile. Due to the small sample size and single-arm trial, the results are not robust, and a large-scale trial is needed to confirm the benefits. However, it provides preliminary clinical and microenvironmental evidence for future trial designs.

Abstract:
Line 58: Hepatic impairment is not in Table 3. Elevated ALT and AST may occur in the same patients. It is unreasonable to sum the patients as if the events occurred at different individuals.

Introduction:
Line 75: Though the PACIFIC regimen is well known among lung cancer experts, it is recommended to express the entire terms for the first appearance of the abbreviation in the article.

Line 101: Please include information on your pre-specified primary endpoint.

Results:
Line 113: Stage N2 is 25 (49.0%) in Table 1, not 26 (47.1%).

Line 120: Is the distribution of the treatment cycles normal? If not, reporting Median is more appropriate since the result reported range instead of standard deviation.

Line 157: The issue is the same as Line 58.

Line 158: Thrombocytopenia, neutropenia, and lymphocytopenia may be observed in the same patients. My concern is the same as hepatic impairment, and so are gastrointestinal symptoms.

Line 172: Supplementary Figure 1e showed p=0.047 for smoking status, and 1f showed p=0.043 for surgery.

Line 173: Please define events. What you described in context does not match supplementary Figure 2.

Discussion:
Line 270: The comparison in the parentheses is inappropriate. The 15.6% was estimated from the subgroup of the full cohort that produced “37.3%.”

Methods:
Line 393: Using 2-tailed in a phase II trial is recommended unless an appropriate rationale is provided. The one-tail 5% is equivalent to two-tail 10%, which is also acceptable in a phase II trial.

Line 440: “Paired t-test” is more appropriate since many tests are pre-and post-tests.

Line 446: OS usually started upon enrollment or randomization in randomized trials. Since this is a single-arm trial, it may begin with enrollment or the treatment starting date. Please explain why the diagnosis date is used as the starting point.

Figure 3a, c: the label of the y-axis is incorrect. The plots showed two box plots for pre- and post-, respectively. The lines between them represented changes. Therefore, the y-axis should be the measurements, not change from the baseline.

Figure 3b: The confidence interval of the two box plots overlapped utterly. Surprisingly, the p-value is so small.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed this reviewer's comments in the response letter. However, only a few of the insightful discussions were incorporated into the revised manuscript. For example, a discussion of spatial transcriptomics and bulk RNA-seq for these valuable samples would be highly beneficial for guiding future research. While the authors briefly touched on the issue of small sample size in the discussion, this reviewer suggests expanding this section to include conclusions drawn from the correlative analysis for added depth and context.

Reviewer #2

(Remarks to the Author)
Although Dr Bian et al nicely responded to the queries raised by the reviewers, a few points should be revised and clarified.

1. Why a new author (Zhida Huang, MD) is included as co-first author? In addition, there is no change in the section of author contribution statement. Please clarify these.

2. Please remove the description of Chinese or CSCO guidelines. Consider the broad readership of Nature Communications, global standard should be maintained.

Reviewer #3

(Remarks to the Author)
The authors addressed most of my comments.
Q10: Showing individual proportions of the full cohort and its subgroup, respectively, is acceptable. After re-reading your sentences, I believe your team had planned four standard cycles of neoadjuvant therapy for the 51 subjects. However, you added two cycles for the subjects who did not achieve the status for surgery. I trust you provide protocol amendments when modifying your treatment plan. If not, I am worried about the rigor of the study. With that, comparing the entire cohort to its subgroup is inappropriate because the subgroup subjects are included in the whole cohort. You are comparing the subjects to themselves, whose results are highly correlated. The p-value is not meaningful. I suggest removing the comparison.

Q11: I accept the explanation. However, the readers may understand the rationale of a one-sided hypothesis if the authors can include their explanation in the manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this interesting study, Dr. Bian and colleagues reported on a single-arm phase II trial evaluating neoadjuvant aumolertinib for unresectable stage III EGFR-mutant non-small cell lung cancer (NSCLC), alongside a correlative/exploratory analysis using bulk RNA-seq.

Reply: Thank you for you review of this study and kind suggestions. Your suggestions will be of great help to refine this study.

What is more, we have also revised the concerning contents in the manuscript according to your suggestions, hoping to make this study more concise and rigorous. We hope the revisions now adequately address your concerns regarding the originality of our work. We are grateful for your insightful feedback and remain open to any further suggestions you may have.

Thank you again for your time and consideration.

Q1: From a clinical perspective, the treatment of locally advanced NSCLC patients with targetable mutations remains a critical challenge. This study showed similar results to previous trials, including a respectable response rate, though the pathologic complete response (pCR) or major pathologic response (MPR) rates were not optimal. For patients with stage III disease, the issue is not solely about tumor resectability but also whether resection will offer a survival benefit. Unfortunately, several studies have shown that surgery after neoadjuvant TKI treatment may not provide additional survival benefits. In other words, even if neoadjuvant TKI converts unresectable cases to resectable ones, surgical resection may not lead to improved outcomes. From this standpoint, this study adds more data to the field but does not provide conceptually novel insights.

Reply 1: We sincerely appreciate your valuable feedback and the time you have taken to review our manuscript. We acknowledge your concerns regarding the novelty of our work, and would like to take this opportunity to further clarify the contributions and innovations presented in our study.

Thank you again for your guidance. The standard treatment for resectable NSCLC harboring *EGFR* mutations (NSCLCm) is concurrent/sequential chemoradiotherapy (cCRT/sCRT) followed by radical

surgery and received long-term *EGFR*-TKI maintenance, or direct surgery followed by long-term *EGFR*-TKI maintenance.

As you mentioned above, the similar studies, including (CTONG-1103[1], NEOS[2], NORA[3], NCT03433469[4]), were enrolled patients with resectable stage III NSCLCm who received neoadjuvant *EGFR*-TKI therapy for 6-8 weeks. These studies explored whether neoadjuvant target-therapy can improve recurrence-free survival (RFS) and overall survival (OS) compared with traditional neoadjuvant chemotherapy. **However, in this trial, participants with unresectable stage III NSCLCm were enrolled**. Before the results of LAURA[5] published, the treatment of unresectable stage III NSCLC does not distinguish the status of driver gene mutations, which does not conform to the principles of precise treatment at the current stage and urgently needs to be reformed. Immune maintenance therapy after cCRT/sCRT was the standard treatment mode for such patients. After the LAURA published, *EGFR*-TKI maintenance therapy after cCRT/sCRT seems to be another option for patients with unresectable stage III NSCLCm. Therefore, this trial used induction *EGFR*-TKI therapy for unresectable stage III NSCLCm to explore the safety and efficacy in the treatment of such patients, trying to provide high efficient and low toxicity treatment for such patients, and provide unresectable NSCLCm with the opportunity of radical surgery. **Besides, in this trial, the enrolled participants received neoadjuvant *EGFR*-TKI for 16 weeks in schedule, and compared to other studies, the objective response rate of tumors remained at a relatively high level.** In the context where *EGFR*-TKI therapy has been proven to provide survival benefits for advanced stage patients and resectable locally advanced patients, **moreover, it is worth exploring whether this therapeutic regimen can create surgical opportunities and provide survival benefits for unresectable NSCLCm in stage III.**

In China, the unresectable NSCLC harboring EGFR mutation in stage III account for a very high proportion of lung cancer patients. Under the background of China's huge population base and high incidence of lung cancer, the diagnosis and treatment of these patients face a huge burden. According to NCCN guidelines, PACIFIC regimen is the first choice for these patients. At the same time, considering the tolerance of Chinese patients to cCRT, as well as the results of the other trial [6], the guidelines of Chinese Society of Clinical Oncology (CSCO) recommend that PACIFIC regimen, or sequential chemoradiotherapy (sCRT) could be performed for the patients. However, on the basis of the poor efficacy of standard treatment (cCRT/sCRT followed by immune-therapy) at this time and the innate insensitivity of immune-therapy (PD-1/L1) for EGFR-mutant NSCLC, how to improve the efficacy of

these patients is a huge and non-negligible challenge. A clinical trial (LAURA, NCT03521154[5]) published in the NEJM has introduced the third-generation EGFR-TKI into the maintenance treatment after cCRT/sCRT for EGFR-mutant NSCLC patients in stage III, significantly improving their prognosis compared to patients receiving cCRT/sCRT alone. **Apart from these, the CSCO guidelines also recommended that neoadjuvant treatment followed by radical surgery could be performed for the patients.**

This trial brings new insights by neoadjuvant/induction target-therapy for stage III unresectable EGFR-mutant NSCLC according to CSCO guidelines, firstly. Besides, we assess the feasibility of neoadjuvant target-therapy followed by radical surgery for these unresectable patients converted into operable ones, which were not explored or fully developed in prior studies. We understand that the initial submission may not have sufficiently emphasized these points, and we appreciate your understanding. In response to your comments, we have revised the manuscript to more clearly highlight these novel aspects, which the goal of ensuring that unique contributions of our study are clearly communicated, particularly in the "Introduction", "Results", and "Discussion" sections. Thank you for your comments again, I hope my explanations might answer at least a part of your concerns. We have supplemented the concerning contents into revision manuscript in the red font:

Page 5, Line 76-83: The latest National Comprehensive Cancer Network (NCCN) guideline recommended that Durvalumab (anti-PD-L1 antibody) after concurrent chemoradiotherapy (cCRT) (PACIFIC regimen) could be performed for unresectable stage III NSCLC harboring *EGFR* mutation (NSCLCm). In the guideline of Chinese Society of Clinical Oncology (CSCO), unresectable stage III NSCLCm could receive PACIFIC regimen or Sugemalimab (anti-PD-L1 antibody) after concurrent/sequential chemoradiotherapy (cCRT/sCRT) (GEMSTONE-301 regimen). Even neoadjuvant/induction therapy followed by radical surgery could be performed for these patients.

Q2: Additionally, the majority of patients with stage III EGFR-mutant NSCLC are not curable. Current guidelines recommend chemoradiotherapy followed by lifelong TKI therapy. The 48-week TKI duration used in this study may not represent the most appropriate timeframe for these patients.

Reply 2: We sincerely appreciate your valuable feedback and the time you have taken to review our manuscript.

At the same time, we apologize for our lack of rigor in the trial process. The research was applied

for approval in our center in December, 2020. At that time, the third-generation EGFR-TKI could not be approved for adjuvant treatment for EGFR-mutant patients. However, this trial was launched in December, 2021. According to the results of the ADAURA trial, we recommend that all surgical treated participants in this trial receiving adjuvant target-therapy for at least 3 years or until the disease progresses. In this trial, among 23 surgical treated patients, 2 of them refused adjuvant target-therapy, and occurred recurrence. In the remaining 21 patients, only 2 of them (2/21, 9.5%) discontinued adjuvant therapy without following the recommended regimen (ADAURA mode). Fortunately, there was no recurrence in both 2 patients. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page: 8, Line: 143-144: There were 19 (82.6%) patients received long-term adjuvant target-therapy or until the disease progresses.

Page: 20, Line: 404-406: Adjuvant Aumolertinib treatment could be administrated within 8 weeks postoperatively. We strongly recommend that the participants continue long-term adjuvant therapy for at least three years or until the disease progresses.

Q3: From a translational perspective, the neoadjuvant treatment/surgery approach offers an advantage over chemoradiotherapy because it provides the opportunity to study cancer evolution under treatment. I would like to commend the authors for successfully collecting and analyzing paired pre- and post-treatment specimens, which are invaluable for understanding cancer evolution. However, two main caveats stand out in this study. First, the small sample size is a significant limitation. When analyzing pre-treatment vs. post-treatment, response vs. non-response, and exon 19 vs. exon 21 mutations, the sample size becomes too small to draw definitive conclusions. Even when statistical differences are observed, confounding factors could be at play.

Reply 3: We deeply appreciate the reviewer's thoughtful concerns about the potential limitations posed by our small sample size and greatly value your insightful critique of our methodology and data analysis. To address the concern you highlighted regarding sample size and technical constraints, we would like to provide additional clarification:

Acquiring tumor samples for sequencing analyses both pre- and post-treatment in EGFR-TKI-related studies presents a substantial challenge, as evidenced in several renowned clinical trials. For

example, while the FLAURA2, ARCHER 1050, and EURTAC studies offered pivotal insights into the efficacy and clinical applications of EGFR-TKI therapies, they did not delve into the transcriptomic characteristics or genomic alterations of TME before and after treatment. This limitation is largely attributable to the inherent constraints of clinical trial designs, particularly the difficulty of collecting treatment-specific tumor samples.

Our study encountered similar challenges in sample collection, particularly in patients with unresectable locally advanced NSCLC (stage III NSCLC). The clinical condition of these patients, combined with the anatomical location of the tumors and the infeasibility of surgical intervention, significantly restricted tumor sample acquisition. Furthermore, the small biopsy specimens were prioritized for pathological and molecular diagnostic purposes, leaving only a minimal amount of material for RNA-seq analyses, and making spatial transcriptomic analyses unfeasible. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts.

Q4: Second, while bulk RNA-seq offers valuable insights, it is limited in scope. For such precious samples, spatial transcriptomics may have provided a much more comprehensive analysis.

Reply 4: Thank you for your comments. We think these are excellent suggestions and comments.

Our study encountered similar challenges in sample collection, particularly in patients with unresectable locally advanced NSCLC (stage III NSCLC). The clinical condition of these patients, combined with the anatomical location of the tumors and the infeasibility of surgical intervention, significantly restricted tumor sample acquisition. Furthermore, the small biopsy specimens were prioritized for pathological and molecular diagnostic purposes, leaving only a minimal amount of material for RNA-seq analyses, and making spatial transcriptomic analyses unfeasible.

Despite these limitations, our RNA-seq analysis provided valuable insights into the remodeling effects of Aumolertinib on the TME, particularly through the marked activation of immune-related genes and pathways, with more pronounced effects observed in patients with Ex19-Del mutations. Your comments have underscored the importance of spatial transcriptomic analyses in furthering our understanding of Aumolertinib's mechanisms of action. We are confident that future investigations, supported by larger sample sizes and integrative multi-omics approaches—particularly spatial transcriptomics—will provide a more comprehensive understanding of TME remodeling and the associated molecular mechanisms.

Reviewer #2 (Remarks to the Author):

Dr Bian et al evaluated treatment and translational outcomes of neoadjuvant aumolertinib in 51 patients with unresectable stage III EGFR-mutant NSCLC. They demonstrated ORR of 70.6%, surgical conversion rate of 45.1%, MPR rate of 21.7%, and pathologic CR rate of 13.0%. In addition, they found increased CD8+ T cells in the post-treatment tissues of responders. Several issues should be clarified.

Reply: We feel grate thank you for your professional review work on our article. As you are concerned, there are several problems that need to be addressed. Your suggestions will be of great help to refine this study. What is more, we have also revised the concerning contents in the manuscript according to your suggestions, hoping to make this study more concise and rigorous.

Q1. Regarding the endpoint, ORR is the rational endpoint of neoadjuvant therapy in EGFR-mutant NSCLC? This should be clarified. In addition, published ORRs of neoadjuvant EGFR TKIs ranged from 42.0% to 81.0% (mostly around 50% including neoadjuvant osimertinib, 52%; PMID: 39028931). Authors should discuss and compare other ORRs, MPR, pathologic CR, and surgical conversion rates in prospective studies.

Reply 1: We appreciate your thoughtful review and constructive feedback.

In our opinions, *EGFR*-TKI suppresses the activity of lung cancer instead of killing the tumor cells as immunotherapy or chemotherapy. The relative low rate of MPR and pCR has been observed in nearly all prospective clinical trials and retrospective analysis, regardless of whether participants receiving the first-generation, the second-generation, or the third-generation *EGFR*-TKI. In the limited number of clinical trials concerning neoadjuvant *EGFR*-TKI therapy, most trials take ORRs as their primary endpoint.

As mentioned above, due to the anti-tumor mechanism of *EGFR*-TKI, there is no significant difference in the pathological response (MPR) rate of NSCLCm after the treatment of different generations *EGFR*-TKI at this stage, which are all relatively low (range from 5%-25%) [4, 7, 8, 9]. On one hand, as the prospective single-arm trial you mentioned using MPR as the primary endpoint (PMID: 39028931) [4], such trial design may lead to negative results in the study. On the other hand, the sample

size required for the trial may be huge as the ongoing NeoADAURA trial [10]. For these 2 reasons, the design of applying MPR as the primary endpoint in phase II clinical trials (proof of concept) needs to be cautious. **Therefore, the previous trials and this trial choose radiological response rate (ORR) as the primary endpoint, and the pathological response rate (MPR/pCR) and survival rate (EFS/OS) as secondary endpoints**.

Thank you again for your kindly suggestions. In the discussion part of revised manuscript, we added the discussion concerning the reasons related to different ORR and MPR among different trials. As we all know, the therapeutic efficacy of *EGFR*-TKI for EGFR-mutant patients is related to the mutation subtypes. Compared with Exon21-L858R patients, Exon19-Del patients have a higher ORR. In addition, the ORR of *EGFR*-TKI is significantly related to the duration of treatment cycle. In the trial you mentioned above, after 8 weeks of neoadjuvant treatment, the ORR was 52% [4]. However, in this trial and previous research in our center [9], 16 weeks of neoadjuvant *EGFR*-TKI therapy can significantly improve the ORR to 70%. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page: 14, Line: 276-287: The trial achieved the pre-specified primary endpoint (ORR: 70.6%), which has potential advantages over the published results of clinical trials previously. This phenomenon might be caused by the following reasons. Firstly, the third-generation *EGFR*-TKIs have demonstrated significantly higher ORR compared to the first-generation *EGFR*-TKIs and traditional chemotherapy for NSCLCm in advanced stage [11, 12, 13]. This suggests that the third-generation *EGFR*-TKIs might be more effective in creating opportunities for radical resection in patients with unresectable NSCLCm. Secondly, the number of neoadjuvant treatment cycles is a crucial issue for unresectable stage III patients. This trial was designed for initial unresectable stage III participants to receive neoadjuvant target-therapy for 16 weeks, which was significantly longer than other studies [2, 4]. However, prolonging the duration of neoadjuvant therapy might improve the incidence of *EGFR*-TKIs resistance and the difficulty of surgical resection potentially. Thus, the optimal number of therapeutic cycles requires further discussion.

Page: 15, Line: 297-301: In this trial, comparing participants with Ex21-L858R mutation, Ex19-Del mutant participants have significantly higher ORR on radiological evaluation (91% vs 59%, $p = 0.006$), and significantly lower mean percentage of residual tumor cells on pathological evaluation (19.8% vs

63.5%, $p = 0.015$) after neoadjuvant Aumolertinib treatment.

Q2. Although PACIFIC trial included EGFR-mutant NSCLC cases, guideline did not support the use of consolidation checkpoint blockade after concurrent chemoradiotherapy. This should be revised in the introduction.

Reply 2: Thank you for your comments. We think these are excellent suggestions and comments. Indeed, due to the high heterogeneity of unresectable NSCLC in stage IIIB-IIIC, the optimal therapeutic regimens for this disease are still unclear. **It is worth mentioning that our research team has conducted a large number of clinical trials and translational researches in the field of neoadjuvant immune-therapy/targeted-therapy for stage III unresectable NSCLC, and has made some progress**. The results have been published in well-known journals in the field of thoracic surgery and oncology (Cell, 2024 [14]; Signal Transduction and Targeted Therapy, 2024 [15]; Nature Communications, 2023 [9]; Genome Medicine, 2023 [16], BMC Medicine, 2022 [17]; Annals of Thoracic Surgery, 2022 [18]).

In China, the unresectable NSCLC harboring EGFR mutation in stage III account for a very large proportion of lung cancer patients. Under the background of China's huge population base and high incidence of lung cancer, the diagnosis and treatment of these patients face a huge burden. Since PACIFIC trial do not exclude the patients harboring EGFR sensitive mutations, concurrent chemoradiotherapy (cCRT) followed by anti-PD-L1 antibody maintenance therapy can also be applied to EGFR mutant unresectable NSCLC in stage III. According to NCCN guidelines, PACIFIC regimen is the first choice for these patients. Compared with stage III NSCLC patients harboring EGFR mutation receiving concurrent chemoradiotherapy (cCRT) alone, PACIFIC regimen could improve the prognosis for unresectable stage III EGFR-mutant patients statistically. At the same time, considering the tolerance of Chinese patients to cCRT, as well as the results of the other trial [6], the CSCO guidelines recommend that PACIFIC regimen, or sequential chemoradiotherapy (sCRT) could be performed for the patients. However, on the basis of the poor efficacy of standard treatment (cCRT/sCRT followed by immune-therapy) at this time and the innate insensitivity of immune-therapy (PD-1/L1) for EGFR-mutant NSCLC, how to improve the efficacy of these patients is a huge and non-negligible challenge. A clinical trial (LAURA, NCT03521154[5]) published in the NEJM has introduced the third-generation EGFR-TKI into the maintenance treatment after cCRT/sCRT for EGFR-mutant NSCLC patients in stage III, significantly improving their prognosis compared to patients receiving cCRT/sCRT alone. **Apart from**

these, the CSCO guidelines also recommended that neoadjuvant treatment followed by radical surgery could be performed for the patients.

This research brings new insights by neoadjuvant/induction target-therapy for stage III unresectable EGFR-mutant NSCLC according to CSCO guidelines, firstly. Besides, we assess the feasibility of neoadjuvant target-therapy followed by radical surgery for these unresectable patients converted into operable ones.

We understand that the initial submission may not have sufficiently emphasized these points, and we appreciate your understanding. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 5, Line 76-83: The latest National Comprehensive Cancer Network (NCCN) guideline recommended that Durvalumab (anti-PD-L1 antibody) after concurrent chemoradiotherapy (cCRT) (PACIFIC regimen) could be performed for unresectable stage III NSCLC harboring *EGFR* mutation (NSCLCm). In the guideline of Chinese Society of Clinical Oncology (CSCO), unresectable stage III NSCLCm could receive PACIFIC regimen or Sugemalimab after concurrent/sequential chemoradiotherapy (cCRT/sCRT) (GEMSTONE-301 regimen). Even neoadjuvant/induction therapy followed by radical surgery could be performed for these patients.

Q3.1 Regarding whole-transcriptome data, authors should evaluate the TCGA immune signature (PMID: 29628290).

Reply 3.1: We are deeply grateful to the reviewer for their constructive suggestions.

The study you referenced (PMID: 29628290) is a significant exploration into TME classification, particularly in advancing our understanding of the relationship between immune subtypes and tumor immune characteristics. Nevertheless, while attempting to reproduce the six immune subtypes described in the study, we encountered several technical obstacles, which largely stem from the insufficient detail provided in the original publication. These challenges are outlined below:

(1) Unclear definitions of immune subtypes

The classification of the six immune subtypes in the referenced study relies on five representative gene modules as core references. However, the subtypes are not defined by the mere high or low expression of a single module but rather by the integrated patterns of multiple modules within each

subtype. Unfortunately, the article does not delineate the specific gene composition of each module or clarify which genes play pivotal roles in subtype definition. This lack of detailed information introduces significant uncertainty when attempting to replicate the subtypes based on gene module characteristics.

(2) Insufficient implementation details for ssGSEA

The article references the use of ssGSEA to score individual gene modules but does not provide details on the scoring range or distribution intervals corresponding to each immune subtype. Furthermore, key parameters for implementing ssGSEA, such as the weighting of gene sets and the method of score normalization, are not explicitly described. These omissions hinder accurate replication of the six immune subtypes outlined in the study.

(3) Incomplete details of the clustering methodology

The study employed the mclust method for finite Gaussian mixture model clustering. However, the article does not specify the approach used to calculate sample distances (e.g., Euclidean distance or Manhattan distance). Moreover, mclust allows for multiple Gaussian mixture models (e.g., diagonal or full covariance matrix models), yet the study does not clarify which model was used. The absence of these technical details further complicates the reproducibility of the immune subtype classification methodology.

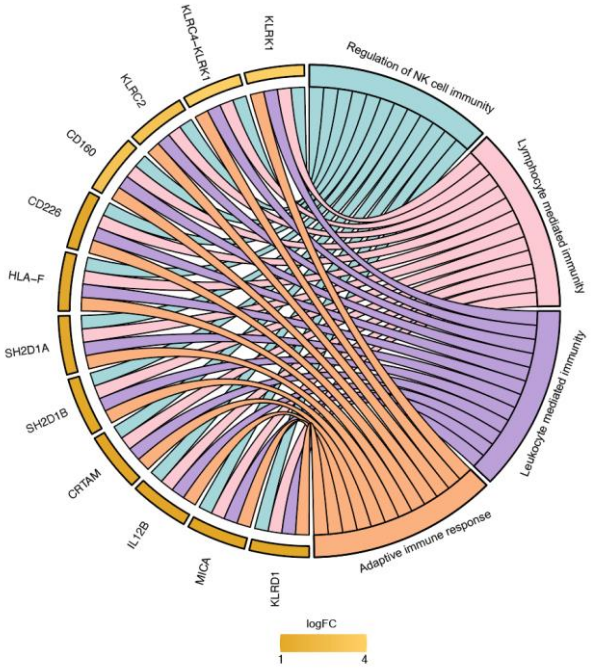
While these ambiguities pose challenges for subsequent analyses based on the immune subtypes proposed in the referenced study, we fully acknowledge its important contributions to the field of immune classification. To address these challenges, we opted to utilize the four TME subtypes proposed by Alexander Bagaev et al. (Fig. 4e). This classification system offers significant advantages, including explicitly defined signature genes for each subtype and a clear delineation of gene module composition and characteristics. By adopting the TME subtypes defined by Bagaev et al., we aim to ensure the reproducibility of our findings while facilitating a more robust investigation into the immune alterations in the tumor microenvironment induced by Aumolertinib treatment.

Q3.2 In addition, the comparison of pre-treatment and post-treatment transcriptome should be explored using Shankey diagram and GOplot correlation between tumors and TME.

Reply 3.2: We sincerely thank the reviewer for your thoughtful and constructive suggestions, which have greatly guided our efforts to further explore and visualize the dynamic transcriptomic changes in tumors and the TME before and after treatment.

In response to your feedback, we have performed additional analyses using GOplot and generated

the corresponding visualizations (Supplementary Fig. 3a). The GOplot analysis effectively highlights the interactions between differentially expressed genes and multiple immune-related pathways, offering a comprehensive and systematic perspective on the remodeling of the TME induced by Aumolertinib treatment.



Supplementary Fig. 3a

With regard to your suggestion of employing Sankey diagrams, we fully agree on their academic value in visually presenting the dynamic changes in tumors and the TME across different treatment stages. However, given the specific conditions of our study and the nature of the available sample data, we faced several constraints that limited the feasibility of implementing Sankey diagrams at this stage. These constraints are detailed below:

(1) Constraints due to the limited number of paired samples

The number of paired pre- and post-treatment samples in this study was inherently limited by practical challenges in clinical operations. In patients with unresectable locally advanced NSCLC (stage III NSCLCm), tumor sample collection is often constrained by factors such as the patient's clinical condition, the tumor's anatomical location, and the infeasibility of surgical procedures. These limitations resulted in a very restricted number of usable samples. Furthermore, some paired samples failed to meet the quality standards for transcriptomic analysis due to RNA degradation, technical issues during sample

processing, or insufficient tissue quantity, which further reduced the number of samples available for paired analysis.

(2) Alternative approaches for dynamic analysis

Although we were unable to implement Sankey diagrams under the current conditions, we conducted in-depth analyses using alternative approaches to explore the dynamic changes in the TME before and after treatment. These analyses revealed significant enrichment of key pathways associated with NK cells, T cells, and adaptive immunity following treatment. Additionally, our findings demonstrated a notable increase in the infiltration of immune cells, including CD8+ T cells, NK cells, and myeloid dendritic cells, after treatment. These results provide evidence for the remodeling effects of Aumolertinib on the TME. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts.

Q4. Authors should clarified unknown EGFR mutation in the Table 1. Authors can evaluate EGFR mutation subtype using RNA-seqeuncing data.

Reply 4: We sincerely thank you for careful reading.

We apologize for your misunderstanding caused by the unclear statement in the previous version of the manuscript. In Table 1 and the article, we mentioned that there were two participants enrolled in this trial harboring EGFR uncommon mutations (ID: AML-005 and AML-030), and we complemented this information into the Supplementary Table 1. We hope this modification can make the description of this article more accurate.

Supplementary Table 1. The characteristics of patients with uncommon *EGFR*-mutant subtypes (N =2)

ID	Gender	Age	ECOG	Smoking	Mutation	TNM	Cycle	c-Regression			Surgery
								RECIST	Primary Tumor	Node	
005	Male	52	0	Never	S768I	IIIB (N2)	3	SD	Shrink 4%	SD	Without
030	Male	50	0	Ever	G719X	IIIB (N2)	2	PR	Shrink 32%	SD	Without

Q5. Do authors evaluate the ctDNA dynamics of pre- and post-treatment settings? If possible, please provide the ctDNA data for this population.

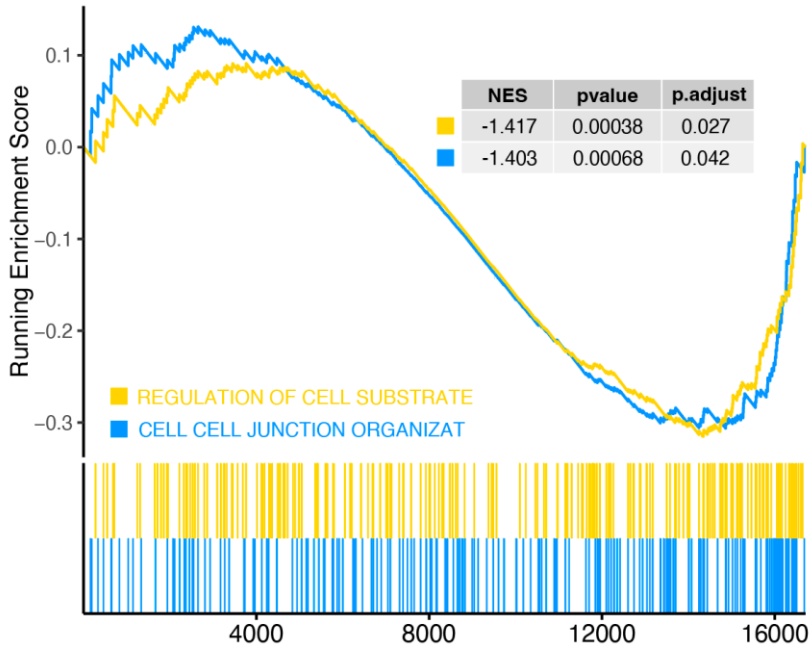
Reply 5: Thank you for your suggestions and comments.

In China, the cost of ctDNA test is high, and it has not been included in national medical insurance, so this test is not routinely tested in clinical work. Thank you for your constructive comments. We will supplement this content to the future trials design to broaden the research horizon and deepen the research content, trying to improve the quality of trials.

Q6. Therapy-induced evolution of oncogene-driven lung cancer is very important. In surgical samples, cell states may be "residual disease" based on the published data (PMID: 32822576). In addition, drug-persister state may exist in post-surgical samples regardless of response states. Please analyze these.

Reply 6: We deeply appreciate your insightful comments regarding the analysis of postoperative residual tumor lesions, which have undoubtedly expanded our understanding of TME dynamics and the plasticity of tumor cells following therapy. As you astutely highlighted, the plasticity of tumor cells and their adaptive evolutionary responses to treatment are critical yet often underexamined aspects, alongside the plasticity of the TME itself.

To address this, we sought to explore potential alterations in residual tumor lesions using bulk RNA-seq data (**Supplementary Fig. 3a**).



Supplementary Fig. 3b

Intriguingly, we observed marked downregulation of pathways such as “Cell Junction

Organization" and "Regulation of Cell Substrate Adhesion" in post-treatment samples. The suppression of these pathways suggests weakened cell-cell junctions and a loss of cell polarity within the tumor microenvironment, potentially facilitating tumor cell detachment from the primary site and promoting enhanced migratory and invasive behavior. These molecular shifts may represent the intricate interplay between tumor cells and the surrounding microenvironment under therapeutic pressure, particularly during adaptive and escape processes. Your thoughtful suggestion has further underscored the need to consider the potential "double-edged sword" effect of targeted therapies. While such treatments effectively eliminate therapy-sensitive tumor cells, they may simultaneously drive adaptive evolution in residual tumor cells, conferring greater migratory and invasive potential. This underscores the critical importance of surgical intervention following medical therapy to eradicate these highly invasive residual cells.

Regrettably, due to limitations in sample collection and technological constraints, we were restricted to bulk RNA-seq data for this analysis. While bulk RNA-seq is highly effective in capturing global gene expression patterns, its inherent limitations in resolving cellular heterogeneity preclude the precise dissection of signals originating specifically from tumor cells or other distinct cellular populations. As a result, conducting an in-depth analysis of the dynamic characteristics of postoperative residual lesions presented a significant challenge within the current study. Nevertheless, we wish to emphasize that the analysis of postoperative residual lesions and drug persistence was not among the primary aims of this study and falls outside the intended scope of the current dataset.

We sincerely thank you for your constructive feedback, which has not only helped us recognize the limitations of our study but has also provided invaluable guidance for shaping future research directions. Moving forward, we will remain committed to exploring this vital area through more comprehensive and technically advanced approaches.

Reviewer #3 (Remarks to the Author):

This trial explored the efficacy and safety of neoadjuvant Aumolertinib for unresectable stage III EGFR-mutant NSCLC. It revealed that 23 subjects proceeded to surgery among the 51

patients enrolled, with an acceptable safety profile. Due to the small sample size and single-arm trial, the results are not robust, and a large-scale trial is needed to confirm the benefits. However, it provides preliminary clinical and microenvironmental evidence for future trial designs.

Reply: Thank you for your kind suggestions. We indeed should have supplemented and improved the statistical methods according to your comments to make the description of this manuscript seems more accurate and concise. Thank you for your assistants.

Abstract:

Q1, Line 58: Hepatic impairment is not in Table 3. Elevated ALT and AST may occur in the same patients. It is unreasonable to sum the patients as if the events occurred at different individuals.

Reply 1：We sincerely thank you for careful reading. Thank you for point this out. We are sorry for the inappropriate description of this chapter in the manuscript.

In this regard, we revised the contents related to adverse events shown in the manuscript and listed the number of each adverse events separately (that is, if a participant occurred an adverse event, the number of this adverse events recorded is 1; if the number of the same adverse event for this participant exceeds 1, it is still recorded as 1). Therefore, we adjusted the contents of the Table concerning adverse events. The total incidence of adverse events in the Table is the number of participants with any adverse event occurred/the number of all participants. While, the statistics of the incidence rate of an adverse event is the number of participants with an adverse event/the number of all participants. I hope the revised version can meet your requirements. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 4, Line 59-62: Fatigue (49.0%), increases in alanine aminotransferase concentration (39.2%), and rash (35.3%) were the most common treatment-related adverse events (TRAEs) of any grade. Grade 3/4 TRAEs occurred in 5 patients (9.8%), and no grade 5 TRAE was recorded.

Table 3. Adverse events in neoadjuvant therapy and surgery

Type of Event	No. of Participants		
	Grades 1-4 (%)	Grade 3-4 (%)	SAE (%)
Adverse Event (n = 51)	49 (96.1)	5 (9.8)	4 (7.8)
Fatigue	25 (49.0)	0	0
ALT elevation	20 (39.2)	1(2.0)	1(2.0)
Rash	18 (35.3)	0	0
Hyponatremia	14 (27.5)	0	0
Acrodynia	12 (23.5)	0	0
Stomatitis	11 (21.6)	0	0
Thrombocytopenia	11 (21.6)	0	0
AST elevation	10 (19.6)	1(2.0)	0
Hyperglycemia	8 (15.7)	0	0
Diarrhea	6 (11.8)	0	0
Creatinine elevation	5 (9.8)	0	0
Chest pain	5 (9.8)	0	0
Neutropenia	4 (7.8)	0	0
Blood bilirubin elevation	4 (7.8)	0	0
Stomachache	3 (5.9)	0	0
Anemia	3 (5.9)	0	0
Nausea	3 (5.9)	0	0
Pruritus	3 (5.9)	0	0
Pneumonia	2 (3.9)	1 (2.0)	1 (2.0)
Abdominal pain	2 (3.9)	0	0
Abdominal distension	2 (3.9)	0	0
Headache	2 (3.9)	0	0
Toothache	2 (3.9)	0	0
Lymphopenia	2 (3.9)	0	0
Lightheadedness	2 (3.9)	0	0

Constipation	2 (3.9)	0	0
Hyperuricemia	2 (3.9)	0	0
Thromboembolic event	1 (2.0)	1 (2.0)	1 (2.0)
Ischemia cerebrovascular	1 (2.0)	1 (2.0)	1 (2.0)
Hyperpotassemia	1 (2.0)	0	0
Hypercalcemia	1 (2.0)	0	0
Urinary tract infection	1 (2.0)	0	0
Arrhythmia	1 (2.0)	0	0
Anorexia	1 (2.0)	0	0
Surgical-Related Complications (n = 23)			
Bronchopleural Fistula	1 (4.3)	0	0

Thank you for your comments again, I hope my explanations might answer at least a part of your doubts.

Introduction:

Q2, Line 75: Though the PACIFIC regimen is well known among lung cancer experts, it is recommended to express the entire terms for the first appearance of the abbreviation in the article.

Reply 2：We sincerely thank you for careful reading. Thank you for point this out. We appreciate for you warm work earnestly and hope the correlation will meet with approval. I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 5, Line 76-83: The latest National Comprehensive Cancer Network (NCCN) guideline recommended that Durvalumab (anti-PD-L1 antibody) after concurrent chemoradiotherapy (cCRT) (PACIFIC regimen) could be performed for unresectable stage III NSCLC harboring *EGFR* mutation (NSCLCm). In the guideline of Chinese Society of Clinical Oncology (CSCO), unresectable stage III NSCLCm could receive PACIFIC regimen or Sugemalimab after concurrent/sequential chemoradiotherapy (cCRT/sCRT) (GEMSTONE-301 regimen). Even

neoadjuvant/induction therapy followed by radical surgery could be performed for these patients.

Q3, Line 101: Please include information on your pre-specified primary endpoint.

Reply 3：We sincerely thank you for careful reading. Thank you for point this out. In the latest version of the manuscript, the relevant content was shown in the chapter of "Method-Sample Size Estimation", according to your requirement and comment, we also explained it in the chapter of Introduction to make the manuscript more clearance. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 6, Line 108-110: This trial successfully achieved the pre-specified primary endpoint, with an objective response rate (ORR) of 70.6%, which was higher than the ORR expected at the time of trial design (70.0%).

Results:

Q4, Line 113: Stage N2 is 25 (49.0%) in Table 1, not 26 (47.1%).

Reply 4：We sincerely thank you for careful reading and reviewing. We feel sorry for our carelessness. It is really a giant mistake to the whole quality of our manuscript. In our revision manuscript, we have corrected the mistakes in manuscript. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 7, Line 121-122: Most participants were categorized as having stage N2 disease (25/51, 49.0%).

Q5, Line 120: Is the distribution of the treatment cycles normal? If not, reporting Median is more appropriate since the result reported range instead of standard deviation.

Reply 5：We sincerely thank you for your careful reading and comments. We are sorry for the improper expression of the statistical content in the latest version of the manuscript. According to your comment, we revised the manuscript to make it more rigorous. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have

supplemented the concerning contents into revision manuscript in the red font:

Page 7, Line 128-130: After a median of 4 cycles (range, 2-6 cycles) of neoadjuvant treatment, ORR, the primary endpoint, was 70.6% (95% CI: 58%-84%).

Page 8, Line 151-153: As shown in **Table 2**, there was no difference in the number of neoadjuvant treatment cycles between Ex19-Del and Ex21-L858R participants (median treatment cycle: 4 cycles vs 4 cycles, $p = 0.397$).

Q6, Line 157: The issue is the same as Line 58.

Reply 6：We sincerely thank you for careful reading. Thank you for point this out. We are sorry for the inappropriate description of this chapter in the manuscript. As previously mentioned, we revised the contents related to adverse events shown in the manuscript and Table 3 and listed the number of each adverse events separately. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 9, Line 166-172: The most common TRAE of any grade was fatigue, which occurred in 25 (49.0%) of 51 participants. Increases in alanine aminotransferase concentration (ALT) (20, 39.2%), rash (18, 35.3%), hyponatremia (14, 27.5%), acrodynia (12, 23.5%), stomatitis (11, 21.6%), and thrombocytopenia (11, 21.6%) were also common treatment-related adverse events. Grade 3/4 TRAEs occurred in 5 patients (9.8%), with cases of ALT concentration elevation, aspartate aminotransferase (AST) concentration elevation, pneumonia, thromboembolic event, and ischemia cerebrovascular disease.

Table 3. Adverse events in neoadjuvant therapy and surgery

Type of Event	**No. of Participants**		
	Grades 1-4 (%)	**Grade 3-4 (%)**	**SAE (%)**
Adverse Event (n = 51)	49 (96.1)	5 (9.8)	4 (7.8)
Fatigue	25 (49.0)	0	0
ALT elevation	20 (39.2)	1(2.0)	1(2.0)
Rash	18 (35.3)	0	0
Hyponatremia	14 (27.5)	0	0

Acrodynia	12 (23.5)	0	0
Stomatitis	11 (21.6)	0	0
Thrombocytopenia	11 (21.6)	0	0
AST elevation	10 (19.6)	1(2.0)	0
Hyperglycemia	8 (15.7)	0	0
Diarrhea	6 (11.8)	0	0
Creatinine elevation	5 (9.8)	0	0
Chest pain	5 (9.8)	0	0
Neutropenia	4 (7.8)	0	0
Blood bilirubin elevation	4 (7.8)	0	0
Stomachache	3 (5.9)	0	0
Anemia	3 (5.9)	0	0
Nausea	3 (5.9)	0	0
Pruritus	3 (5.9)	0	0
Pneumonia	2 (3.9)	1 (2.0)	1 (2.0)
Abdominal pain	2 (3.9)	0	0
Abdominal distension	2 (3.9)	0	0
Headache	2 (3.9)	0	0
Toothache	2 (3.9)	0	0
Lymphopenia	2 (3.9)	0	0
Lightheadedness	2 (3.9)	0	0
Constipation	2 (3.9)	0	0
Hyperuricemia	2 (3.9)	0	0
Thromboembolic event	1 (2.0)	1 (2.0)	1 (2.0)
Ischemia cerebrovascular	1 (2.0)	1 (2.0)	1 (2.0)
Hyperpotassemia	1 (2.0)	0	0
Hypercalcemia	1 (2.0)	0	0
Urinary tract infection	1 (2.0)	0	0

Arrhythmia	1 (2.0)	0	0
Anorexia	1 (2.0)	0	0
Surgical-Related Complications (n = 23)			
Bronchopleural Fistula	1 (4.3)	0	0

Q7, Line 158: Thrombocytopenia, neutropenia, and lymphocytopenia may be observed in the same patients. My concern is the same as hepatic impairment, and so are gastrointestinal symptoms.

Reply 7： We sincerely thank you for careful reading. We are sorry for the inappropriate description of this chapter in the manuscript.

As previously mentioned, we revised the contents related to adverse events shown in the manuscript and listed the number of each adverse events separately (that is, if a participant occurred an adverse event, the number of this adverse events recorded is 1; if the number of the same adverse event for this participant exceeds 1, it is still recorded as 1). Therefore, we adjusted the contents of the Table 3 concerning adverse events. I hope the revised version can meet your requirements. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

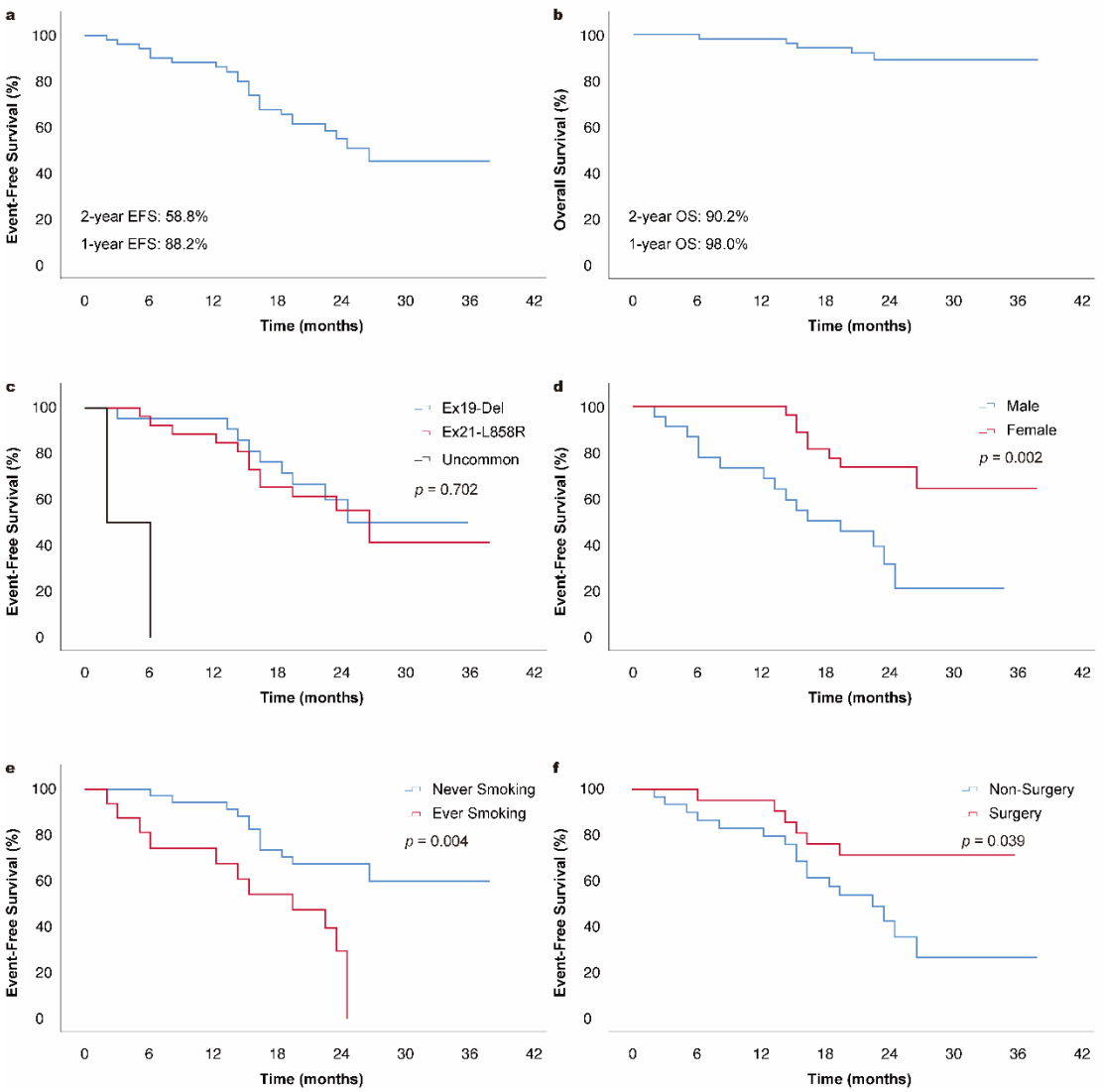
Page 9, Line 166-172: The most common TRAE of any grade was fatigue, which occurred in 25 (49.0%) of 51 participants. Increases in alanine aminotransferase concentration (ALT) (20, 39.2%), rash (18, 35.3%), hyponatremia (14, 27.5%), acrodynia (12, 23.5%), stomatitis (11, 21.6%), and thrombocytopenia (11, 21.6%) were also common treatment-related adverse events. Grade 3/4 TRAEs occurred in 5 patients (9.8%), with cases of ALT concentration elevation, aspartate aminotransferase (AST) concentration elevation, pneumonia, thromboembolic event, and ischemia cerebrovascular disease.

Q8, Line 172: Supplementary Figure 1e showed p=0.047 for smoking status, and 1f showed p=0.043 for surgery.

Reply 8： We sincerely thank you for careful reading. We are sorry for the inappropriate

description of this chapter in the manuscript. In the process of this major revision of this manuscript, we conducted a new round of follow-up work on the participants, with a median follow-up time of 24.0 months up to January 2025. Simultaneously performed calculations on OS and EFS of this trial. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 9-10, Line 179-187: As of the cut-off date of January 1st 2025, the median follow-up duration for all participants was 24.0 months (interquartile range (IQR), 22.0-26.0 months). The one and two-year EFS rate were 88.2% and 58.8% respectively. Besides, the one and two-year OS rate were 98.0% and 90.2% respectively. Female patients ($p = 0.002$), never smokers ($p = 0.004$), and surgical treated patients ($p = 0.039$) had significantly better EFS. No significant difference in EFS was observed between the participants harboring Ex19-Del and Ex21-L858R ($p = 0.702$). While, EFS of sensitive mutant participants demonstrated significantly superior than participants with uncommon mutant subtypes (Ex18-G719X and Ex20-S768I) ($p < 0.001$ for both) (**Supplementary Fig. 1**).



Supplementary Fig. 1

Q9, Line 173: Please define events. What you described in context does not match supplementary Figure 2.

Reply 9: We thank you for reminding us of the improper expression here, and we apologize for our unclear statement misleading your judgment.

In the chapter of Patients and Methods-Statistical Analysis, the EFS was defined as the time between the first dosage of neoadjuvant Aumolertinib and any progression of disease before surgery, disease recurrence after surgery, disease progression in the absence of surgery, treatment strategy changing from any reasons, or death from any cause. If the participants of this trial have several of the above events in turn during the follow-up period, the first event during the follow-up period is defined as the event related to "event-free survival (EFS)". Thank

you for your careful inspection. With your help, the description of this trial report is clearer and more accurate. Thank you for your comments again, I hope my explanations might answer at least a part of your doubts. We have supplemented the concerning contents into revision manuscript in the red font:

Page 23, Line 469-475: EFS was defined as the time between the first dosage of neoadjuvant Aumolertinib and any progression of disease before surgery, disease recurrence after surgery, disease progression in the absence of surgery, treatment strategy changing from any reasons, or death from any cause. If the participants of this trial have several of the above events in turn during the follow-up period, the first event during the follow-up period is defined as the event related to "event-free survival (EFS)".

Discussion:

Q10, Line 270: The comparison in the parentheses is inappropriate. The 15.6% was estimated from the subgroup of the full cohort that produced "37.3%."

Reply 10: We thank you for reminding us of the improper expression here, and we apologize for our unclear statement misleading your judgment.

Indeed, we recognize that there is a relative lack of rigor in comparing the rate of surgical conversion between these two parts of participants, thus we present the results in the Chapter of Discussion and make a relatively reasonable guess. However, for patients with stage III NSCLCm, there is a lack of absolute standard treatment. We are also making certain assumptions for the optimization of trials in the future. For unresectable stage III NSCLCm, compared with the participants who underwent surgery within 4 cycles of neoadjuvant target-therapy, there was no statistical difference in the surgical conversion rate of participants who received more than 4 cycles of neoadjuvant target-therapy because the patients received 4 cycles neoadjuvant therapy could not be converted into operable status (19/51 vs 4/32, p = 0.057). These results indicated that prolonging the duration of neoadjuvant target-therapy extending 4 cycles has limited effect on improving the conversion rate of radical surgery. This is what we want to show in the manuscript. We hope that our explanation can make you known our views more clearly. However, if you still think that the description here is unreasonable, we will delete this part of views in the next revision manuscript. Thank you for your professional

and kind comments again. We have supplemented the concerning contents into revision manuscript in the red font:

Page 15, Line 291-293: OS was defined as the first dosage of neoadjuvant Aumolertinib to death from any cause.

Methods:

Q11, Line 393: Using 2-tailed in a phase II trial is recommended unless an appropriate rationale is provided. The one-tail 5% is equivalent to two-tail 10%, which is also acceptable in a phase II trial.

Reply 11：We sincerely thank you for careful reading. We thank you for your comments about the parameters selected in the calculation of the sample size in this trial.

Indeed, in this proof-of-concept phase II trial, we attempted to evaluate the feasibility of neoadjuvant third-generation EGFR-TKI treatment for stage III unresectable EGFR-mutant NSCLC. By comparing with historical data (EMERGING-CTONG 1103 [1]) and using rigorous design and accurate calculations, a certain number of participants were enrolled.

In my opinion, when we cannot determine the efficacy advantage/disadvantage of the two therapeutic regimens, the two-tailed test should be performed to calculate sample size. However, in previous prospective and retrospective studies concerning target-therapy for NSCLCm, we can clearly understand that **the efficacy of third-generation *EGFR*-TKI in treating NSCLCm is superior than first-generation *EGFR*-TKI**. If the null hypothesis of the statistical test is that the ORR of drug A is not equal to that of drug B, then there are two possible outcomes: the ORR of drug A is greater than that of drug B or the ORR of drug A is less than that of drug B. Therefore, a two-sided test is required to account for both scenarios. If the null hypothesis of the statistical test is that the ORR of drug A is greater than that of drug B, then there is only one scenario, and a one-sided test is sufficient to meet the testing requirements. In this trial, we only aim to explore if the ORR of the third-generation TKI as neoadjuvant settings is larger than the first-generation TKI.

Therefore, we chose to use the “one-tailed” to calculate the sample size of this trial, which might be considered accurate and reasonable. We hope that our explanation can make you known our views more clearly. Thank you for your professional and kind comments again.

Q12, Line 440: “Paired t-test” is more appropriate since many tests are pre-and post-tests.

Reply 12: We sincerely appreciate the reviewer’s valuable suggestion regarding the application of the Paired t-test for statistical analysis. The Paired t-test is indeed a well-established method for analyzing changes in paired samples, as it effectively controls for inter-individual variability and enhances the sensitivity of statistical tests. We fully recognize its robustness and suitability in studies of this nature.

Nevertheless, the primary reason we refrained from utilizing the Paired t-test in this study lies in the substantial limitation of paired sample availability before and after treatment. This limitation stems from several objective challenges inherent to clinical practice. Specifically, in patients with unresectable locally advanced NSCLC (stage III NSCLC), tumor sample collection is often constrained by patients' clinical conditions, tumor localization, and the impracticality of surgical intervention, resulting in a severely restricted number of usable samples. Moreover, additional reductions in paired sample numbers occurred due to RNA degradation, technical complications during sample processing, and insufficient tissue quantity, which rendered certain samples unsuitable for transcriptomic analysis and precluded their inclusion in paired analyses.

Despite these challenges, we employed a comprehensive suite of statistical and bioinformatics tools (e.g., DESeq2, GSEA, MCPcounter) to rigorously assess transcriptomic changes before and after treatment. These methods are widely recognized and have been extensively validated in transcriptomic studies involving similarly constrained sample sizes. Importantly, they provide reliable results even in the absence of paired samples, thereby enabling us to derive credible biological insights from the available data.

Once again, we sincerely thank the reviewer for this thoughtful suggestion. We trust that the explanation provided here elucidates our rationale for not adopting the Paired t-test and demonstrates the scientific rigor and appropriateness of the alternative analytical approaches we employed under the practical constraints of limited sample availability.

Q13, Line 446: OS usually started upon enrollment or randomization in randomized trials. Since this is a single-arm trial, it may begin with enrollment or the treatment starting date.

Please explain why the diagnosis date is used as the starting point.

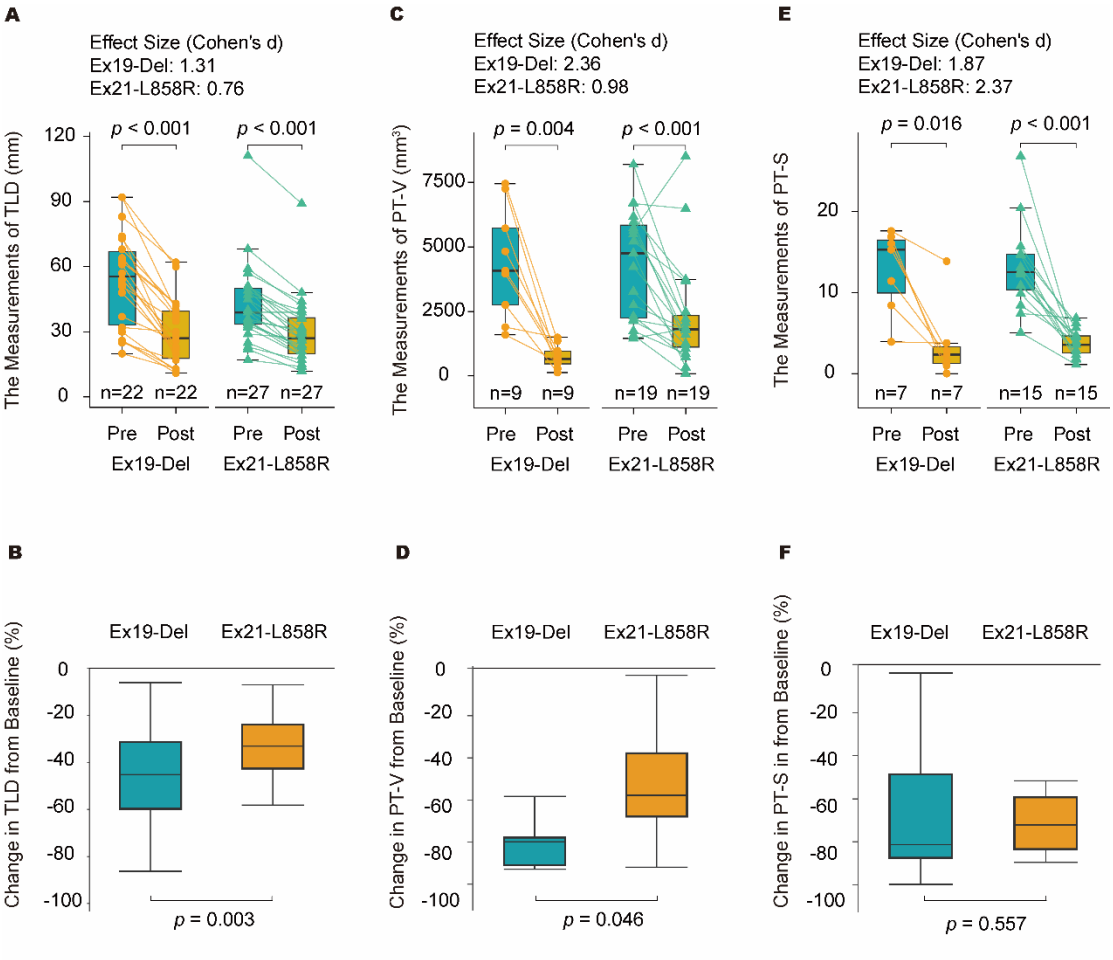
Reply 13: We thank you for reminding us of the improper expression here, and we apologize for our unclear statement misleading your judgment.

Indeed, in our center, the interval time between a patient was diagnosed as *EGFR*-mutant NSCLC and participated in this clinical trial for neoadjuvant target-therapy was very short. Thus, the two time-nodes were not specifically distinguished in the previous version of the manuscript. Thank you for your suggestion. We unified the initial measurement of EFS and OS as the time of receiving the first dosage of neoadjuvant treatment. This change also makes this article more accurate and scientific. We have supplemented the concerning contents into revision manuscript in the red font:

Page 23, Line 474-475: OS was defined as the first dosage of neoadjuvant Aumolertinib to death from any cause.

Q14, Figure 3a, c: the label of the y-axis is incorrect. The plots showed two box plots for pre- and post-, respectively. The lines between them represented changes. Therefore, the y-axis should be the measurements, not change from the baseline.

Reply 14: We thank you for reminding us of the improper expression here, and we apologize for our unclear statement misleading your judgment. According to your comments, we revised the concerning contents in the revised manuscript **Fig 3**. These revisions also make this article more accurate and scientific.



Q15, Figure 3b: The confidence interval of the two box plots overlapped utterly. Surprisingly, the p-value is so small.

Reply 15: We thank you for reminding us here. Through careful inspection and re-calculation, we believe that the statistical results of this item are accurate and reasonable. Thank you again for your suggestions and comments.

Finally, we express sincerely thanks to your nice work again. With your help, we avoided oversights regarding statistical issues, making the contents of this study more informative and the conclusions of this study more reliable.

Reference:

1. Zhong WZ, *et al.* Erlotinib versus gemcitabine plus cisplatin as neoadjuvant treatment of stage IIIA-N2 EGFR-mutant non-small-cell lung cancer: final overall survival analysis of the EMERGING-CTONG 1103 randomised phase II trial. *Signal Transduct Target Ther* **8**, 76 (2023).

2. Lv C*, et al.* Osimertinib as neoadjuvant therapy in patients with EGFR-mutant resectable stage II-IIIB lung adenocarcinoma (NEOS): A multicenter, single-arm, open-label phase 2b trial. *Lung Cancer* **178**, 151-156 (2023).

3. Lee JB*, et al.* Neoadjuvant and adjuvant osimertinib in stage IA-IIIA, EGFR-mutant non-small cell lung cancer (NORA). *J Thorac Oncol*, (2024).

4. Blakely CM*, et al.* Neoadjuvant Osimertinib for the Treatment of Stage I-IIIA Epidermal Growth Factor Receptor-Mutated Non-Small Cell Lung Cancer: A Phase II Multicenter Study. *J Clin Oncol*, Jco2400071 (2024).

5. Lu S*, et al.* Osimertinib after Chemoradiotherapy in Stage III EGFR-Mutated NSCLC. *N Engl J Med*, (2024).

6. Belderbos J*, et al.* Randomised trial of sequential versus concurrent chemo-radiotherapy in patients with inoperable non-small cell lung cancer (EORTC 08972-22973). *Eur J Cancer* **43**, 114-121 (2007).

7. Zhong W*, et al.* EGFRErlotinib Versus Gemcitabine Plus Cisplatin as Neoadjuvant Treatment of Stage IIIA-N2 -Mutant Non-Small-Cell Lung Cancer (EMERGING-CTONG 1103): A Randomized Phase II Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **37**, 2235-2245 (2019).

8. Zhang Y*, et al.* Gefitinib as neoadjuvant therapy for resectable stage II-IIIA non-small cell lung cancer: A phase II study. *J Thorac Cardiovasc Surg*, (2020).

9. Bian D*, et al.* Neoadjuvant Afatinib for stage III EGFR-mutant non-small cell lung cancer: a phase II study. *Nature Communications* **14**, (2023).

10. Tsuboi M*, et al.* Neoadjuvant osimertinib with/without chemotherapy versus chemotherapy alone for EGFR-mutated resectable non-small-cell lung cancer: NeoADAURA. *Future Oncol*, (2021).

11. Soria JC*, et al.* Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* **378**, 113-125 (2018).

12. Shi Y*, et al.* Furmonertinib (AST2818) versus gefitinib as first-line therapy for Chinese patients with locally advanced or metastatic EGFR mutation-positive non-small-cell lung cancer (FURLONG): a multicentre, double-blind, randomised phase 3 study. *The Lancet Respiratory medicine*, (2022).

13. Lu S*, et al.* AENEAS: A Randomized Phase III Trial of Aumolertinib Versus

Gefitinib as First-Line Therapy for Locally Advanced or MetastaticNon-Small-Cell Lung Cancer With EGFR Exon 19 Deletion or L858R Mutations. *J Clin Oncol* **40**, 3162-3171 (2022).

14. Liu Q*, et al.* Proteogenomic characterization of small cell lung cancer identifies biological insights and subtype-specific therapeutic strategies. *Cell* **187**, 184-203.e128 (2024).

15. Xia H*, et al.* Neoadjuvant camrelizumab (an anti-PD-1 antibody) plus chemotherapy or apatinib (a VEGFR-2 inhibitor) for initially unresectable stage II-III non-small-cell lung cancer: a multicentre, two-arm, phase 2 exploratory study. *Signal Transduct Target Ther* **9**, 145 (2024).

16. Hu J*, et al.* Tumor microenvironment remodeling after neoadjuvant immunotherapy in non-small cell lung cancer revealed by single-cell RNA sequencing. *Genome Med* **15**, 14 (2023).

17. Zhu X*, et al.* Safety and effectiveness of neoadjuvant PD-1 inhibitor (toripalimab) plus chemotherapy in stage II-III NSCLC (LungMate 002): an open-label, single-arm, phase 2 trial. *BMC Med* **20**, 493 (2022).

18. Zhang P*, et al.* Neoadjuvant Sintilimab and Chemotherapy for Resectable Stage IIIA Non-Small Cell Lung Cancer. *The Annals of thoracic surgery*, (2022).

Reviewer Comments

Reviewer #1

The authors have addressed this reviewer's comments in the response letter. However, only a few of the insightful discussions were incorporated into the revised manuscript. For example, a discussion of spatial transcriptomics and bulk RNA-seq for these valuable samples would be highly beneficial for guiding future research. While the authors briefly touched on the issue of small sample size in the discussion, this reviewer suggests expanding this section to include conclusions drawn from the correlative analysis for added depth and context.

We are deeply grateful to you for your thoughtful and constructive feedback. Your suggestions have been invaluable in enhancing both the scientific rigor and the clinical relevance of our manuscript. In response, we have made the following revisions:

1. Incorporation of discussions on spatial transcriptomics and bulk RNA-seq for guiding future research

We wholeheartedly agree that discussing spatial transcriptomics would significantly complement our bulk RNA-seq findings. In light of your suggestion, we have carefully revised the Limitations section in the red font (Page: 18, Line: 366-375) to include the following passage:

Revised: Fifth, the bulk RNA-seq analysis lacked paired tumor samples, and future studies should prioritize the collection of paired samples to enable more robust longitudinal analyses. Furthermore, our study faces challenges common to clinical trials of EGFR-TKIs (such as FLAURA2 and ARCHER 1050), where the limited availability of tissue samples in real-world clinical settings hinders the ability to perform multi-omics analyses. Although our bulk RNA-seq data revealed patterns of immune activation, spatial transcriptomics, which maps gene expression within the tissue architecture, could more precisely elucidate the local immune microenvironment and tumor-stroma interactions. In the future, it will be necessary to integrate spatial analysis into single-cell or multi-region sequencing to validate our findings and further dissect the spatial determinants of treatment response.

We hope this addition clearly reflects our recognition of the potential benefits of spatial transcriptomics for guiding future research.

2. Expanded discussion on small sample size and correlative analyses

We sincerely appreciate your recommendation to further discuss the impact of our limited sample size and to incorporate additional insights from our correlative analysis. In response, we have significantly expanded the Discussion section (Page: 15-17, Line: 307-335) to elaborate on these points in the red font:

Revised: In the above context, the results of bulk RNA-seq in this trial demonstrates that Ex21-L858R samples demonstrate a more extensive expression of cell cycle activation-related genes and pathways comparing to Ex19-Del samples. This finding aligns with the research of Zhu et al., which demonstrated that, relative to the L858R mutation, Ex19-Del cells showed enhanced suppression of AKT/ERK phosphorylation and G1 phase arrest upon gefitinib treatment, correlating with improved long-term survival in Ex19-Del patients. Our results extend this paradigm by identifying FBXO43-mediated stabilization of SKP2 as a novel mechanism facilitating cell cycle progression in Ex21-L858R tumor cells following TKI treatment. Specifically, FBXO43 is upregulated in post-treatment Ex21-L858R samples, where AT1 phosphorylation diminishes SKP2 ubiquitination and degradation. SKP2, a known oncogene, facilitates the ubiquitination and subsequent degradation of tumor suppressor substrates. Additionally, we observed an upregulation of the MAPK pathway, which is critically linked to EGFR-TKI resistance, in patients harboring the Ex21-L858R mutation treated with EGFR-TKIs. The differential activation of the cell cycle and MAPK pathways between these two mutant subtypes may constrain the therapeutic efficacy of neoadjuvant Aumolertinib treatment in Ex21-L858R mutation patients.

Moreover, our study uncovers previously unreported immunological differences between the two mutation subtypes: Ex19-Del tumors demonstrate a higher degree of post-TKI immune activation, characterized by elevated CD8+ T cell/myeloid dendritic cell populations, and an upregulation of dMMR compared to Ex21-L858R tumors. This novel finding contrasts with Zhu et al.'s focus on cell-autonomous signaling, and suggests that the superior clinical outcomes observed in Ex19-Del patients may stem from intrinsic signaling vulnerabilities and immune permissiveness. The concurrent upregulation of NER/HDR/NHEJ DNA repair pathways in

Ex19-Del tumors further points to a distinct adaptive DNA repair mechanism that could potentially enhance the efficacy of subsequent immunotherapy, a therapeutic approach not previously considered in studies comparing EGFR mutation subtypes. These insights imply that Ex21-L858R patients may benefit from the earlier incorporation of cell cycle/MAPK inhibitors alongside TKIs, whereas Ex19-Del tumors may derive greater benefit from sequential immunotherapy.

As the frontline therapeutic agents for patients with EGFR mutations, EGFR-TKIs induce dynamic alterations in TME, and the implications of these changes for subsequent immunotherapy remain a matter of debate. Our results from bulk RNA-Seq have elucidated that in patients exhibiting a response to Aumolertinib, there is an upregulation of genes such as IL2, LAT, and KLRK1, which are integral to the proliferation and activation of T cells and NK cells. We also observed an enhancement in lymphocyte, leukocyte, and NK cell-mediated immune pathways. Immune infiltration analysis confirms a notable increase in CD8+ T cells, NK cells, and myeloid dendritic cells post-treatment. Concurrently, cytotoxic molecules and HLA are also elevated, which are closely associated with CD8+ T cells, corroborating the findings of Lu et al., who demonstrated that short-term TKI treatment enhances antitumor immunity by promoting the infiltration of cytotoxic cells. Furthermore, our classification of TME in tumor samples identified a significant upregulation in the Immune-Enriched-Non-Fibrotic subtype, which is most conducive to immunotherapy, following neoadjuvant EGFR-TKIs treatment. Interestingly, PD-1 expression in primary tumors is markedly increased after neoadjuvant EGFR-TKIs treatment. However, between baseline and post-treatment tumor samples, a similar trend could not be observed in PD-L1 expression, especially in the non-response group. Taken together, our observation continues to suggest the potential effectiveness of a sequential treatment regimen involving EGFR-TKIs followed by immunotherapy, particularly in patients with Ex19-Del mutations. By strategically implementing immunotherapy during the period when EGFR-TKIs induce tumor microenvironment activation, it may be feasible to achieve maximal remission for inoperable stage III NSCLCm patients.

We believe that this expanded discussion more thoroughly addresses the limitations of our study and the clinical implications of our correlative analyses.

Once again, we express our heartfelt thanks for your invaluable input. We trust that these revisions adequately address your concerns and further strengthen the manuscript. All changes have been clearly highlighted in the revised version, and we sincerely hope that our responses meet with your approval.

Thank you for your time and constructive guidance.

Reviewer #2

Although Dr Bian et al nicely responded to the queries raised by the reviewers, a few points should be revised and clarified.

1. Why a new author (Zhida Huang, MD) is included as co-first author? In addition, there is no change in the section of author contribution statement. Please clarify these.

Reply 1: Thank you for your careful observation. We have added Zhida Huang as a co-author because, during this round of revisions, Dr. Huang has undertaken a significant amount of work related to patient follow-up, data collection, and statistical analysis, making an important contribution to the improvement of this trial report. We apologize for our oversight in the part of **Author Contribution Statement** and sincerely appreciate your meticulous attention to detail. The relevant content has been highlighted in red font in the revised version (Page: 31, Line: 754).

Revised: P.Z., and D.B. conceived and designed the study. L.J. M.L., W.H., L.D., D.X., X.L., Y.Zhu, X.Z., and J.Z. provided the materials and patients support. G.J., and D.Z. provided the administrative support. D.B., L.Z., C.W., Y.L., S.J., X.B., J.Y., Y.Zhou., Z.H., L.S., J.Hu., J.Huang, and J.L. collected the data. D.B., L.Z., Z.H., Y.L., S.J., L.S., J.Hu., and Y.Zheng. analyzed and interpreted the data. D.B., S.J., L.Z., and J.Hu. wrote the draft of the manuscript. All authors approved the final version of the manuscript for submission.

2. Please remove the description of Chinese or CSCO guidelines. Consider the broad readership of Nature Communications, global standard should be maintained.

Reply 2: Thank you for your valuable suggestions and feedback. We have carefully considered

your recommendation and have revised the manuscript by removing the relevant Chinese guideline-related content. In its place, we have incorporated supporting data from the study (GEMSTONE-301) to strengthen our perspective. This adjustment is intended to enhance the accessibility and relevance of our report for a wider readership. We sincerely appreciate your insightful comments. The relevant content has been highlighted in red font in the revised version (Page: 5, Line: 79-82).

Revised: Additionally, GEMSTONE-301 study demonstrated that, Sugemalimab (anti-PD-L1 antibody) may confer a prognostic benefit for patients with unresectable stage III NSCLC when administered following concurrent or sequential chemoradiotherapy (cCRT/sCRT).

Thank you for your time and constructive guidance.

Reviewer #3

The authors addressed most of my comments.

Q10: Showing individual proportions of the full cohort and its subgroup, respectively, is acceptable. After re-reading your sentences, I believe your team had planned four standard cycles of neoadjuvant therapy for the 51 subjects. However, you added two cycles for the subjects who did not achieve the status for surgery. I trust you provide protocol amendments when modifying your treatment plan. If not, I am worried about the rigor of the study. With that, comparing the entire cohort to its subgroup is inappropriate because the subgroup subjects are included in the whole cohort. You are comparing the subjects to themselves, whose results are highly correlated. The p-value is not meaningful. I suggest removing the comparison.

Reply 1.1: Thank you for your valuable suggestions and feedback. We sincerely apologize for the insufficient explanation in the English translation regarding the latest version of the Study Protocol (V1.1) is as follows:

Patients assessed as inoperable after four cycles of neoadjuvant target-therapy will undergo a multidisciplinary team (MDT) discussion to determine the next phase of treatment. This discussion will involve experts from thoracic surgery, oncology, radiation therapy, and respiratory medicine. The specific decisions are as follows:

- **Patients with Partial Response (PR):** If it is anticipated that continuing the original treatment plan will benefit the patient, the original treatment plan will be maintained.

- **Patients with Stable Disease (SD) or Progressive Disease (PD):** For patients assessed as SD or PD after neoadjuvant therapy, as well as those who develop PD after surgery, they will be transferred to the oncology department for comprehensive treatment. The specific treatment plan will be determined through multidisciplinary discussion and may include, but is not limited to:

1. Continued neoadjuvant target-therapy (for some SD patients);
2. Chemotherapy;
3. Radiotherapy.

The relevant content of Trial Protocol has been uploaded to Journal as Supplementary File. We sincerely appreciate your insightful comments.

Reply 1.2: Furthermore, in accordance with your suggestion, we have removed the comparative discussion regarding the extension of neoadjuvant therapy cycles and have instead proposed a direction for further in-depth research. We sincerely appreciate your guidance, which has enhanced the rigor of the manuscript and the reliability of its conclusions. The relevant content has been highlighted in red font in the revised version (Page: 15, Line: 290-292).

Revised: The potential impact of prolonging neoadjuvant therapy on enhancing the surgical conversion rate among patients with initially unresectable conditions remains an area requiring further investigation.

Q11: I accept the explanation. However, the readers may understand the rationale of a one-sided hypothesis if the authors can include their explanation in the manuscript.

Reply 2: Thank you for your valuable suggestions and feedback. We sincerely appreciate your guidance. The explanation of this statistical content has been added in red font in the revised version (Page: 21-22, Line: 443-446).

Revised: &: If the null hypothesis of the statistical test is that the ORR of drug A is superior to that of drug B, only one scenario needs to be considered, rendering a one-sided test adequate to fulfill the statistical requirements. In this trial, our objective is solely to investigate whether the

ORR of third-generation TKIs in neoadjuvant settings exceeds that of first-generation TKIs.

Thank you for your time and constructive guidance.