Peer Review File

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Reviewer A

Comment 1: Concise and well done.
Reply 1: Thank you for acknowledging our efforts. Your recognition inspires us to pursue even more impactful clinical research.

Reviewer B

Comment 1: There are minor grammatical errors that needs further proofreading (e.g., Line 81-82: sentence incomplete)
Reply 1: Thank you so much for your careful check. The grammatical error here has been corrected in the revised manuscript.
Changes in the text: The statement has been revamped as " However, in the eras of immunotherapy, it is unclear whether long-term use of chemotherapy is needed as recommended in the 2021 Chinese Society of Clinical Oncology (CSCO) guidelines." (see Page 4, line 97-99).

Comment 2: Since this study compared 4 groups simultaneously, and the actual N = 140 only, please indicate your power calculation & level of confidence. The subsequent method of comparison (e.g., ANOVA) needs to be mentioned in the method.
Reply 2: Thank you for your thorough review and the insightful suggestions regarding the statistical aspects of our study. As our investigation is based on retrospective data, it was not feasible to conduct a priori power calculations before initiating the study. Our sample size was determined by the number of patient records available during the study period, which is characteristic of retrospective research designs, thus inherently defined by the scope of the accessible dataset.

In such research designs, power analysis is typically not a pre-planned component but rather more commonly applied to sample size planning in prospective, randomized controlled trials. We acknowledge that a post hoc power analysis could offer some insights; however, given its interpretive limitations and the specific context of our study, we have decided against conducting such an analysis.

Our analytical approach was to use log-rank analysis to compare the prognostic differences between the various maintenance treatment regimens. We have detailed this approach within the methods section and have reported the corresponding levels of statistical significance and confidence intervals in the results section to ensure the transparency and interpretability of our findings.

We believe that our study results still provide valuable insights that can guide future research directions and patient treatment decisions, even in the absence of a priori power analysis.

Comment 3: For Table 1, please indicate whether (and which) baseline characteristics are statistically different in between the four groups.
Reply 3: Thank you for your comment regarding the inclusion of statistical comparisons of baseline characteristics across the four groups in our study. In response to your suggestion, we have conducted a thorough comparison of the baseline characteristics and have included the p-values for these comparisons in the revised Table 1. The majority of baseline characteristics among participants in all four groups were similar, with the exception of histologic features and PD-L1 expression. We have made revisions in our methods and results.
Changes in the text: we added the statement as "In order to evaluate the baseline characteristics and assess the comparability of the four groups, continuous variables were analyzed using one-way ANOVA (Analysis of Variance) when they were normally distributed, or the Kruskal-Wallis test for data not meeting the assumptions of normality. Categorical variables were analyzed using the Chi-square test or Fisher's exact test." (see Page 6 line 177-181)
"The majority of baseline characteristics among participants in all four groups were similar, with the exception of histologic features and PD-L1 expression." (see Page 6 line 197-199)
We added p value in revise Table 1.

Comment 4: For figure 2A, please include the HR (as included in the text ~ Line 184 - 192)
Reply 4: Thank you for your valuable feedback regarding Figure 2A. As per your suggestion, we have now included the Hazard Ratio (HR) in the revised Figure 2A, consistent with the details provided in the text on lines 184 to 192. This addition provides a visual representation of the HR, complementing the textual description and allowing for an immediate and clear understanding of the magnitude and direction of the effect observed in our study.
Changes in the text: we included the Hazard Ratio (HR) in the revised Figure 2A (see Figure 2A)

Comment 5: For the chemo-free period calculation (Line 19 - 195; Figure 2B), please indicate if the differences were statistically significant.
Reply 5: Thank you for suggestion. We have addressed your question by adding the p-values to the respective sections of the text.
Changes in the text: We added "(p=0.038)" and "(p=0.773)" (see Page 6 line 211, line 212).

Comment 6: Similarly, was the proportion of PR/SD/CR/PR between the four arms statistically different? (Figure 3, Line 196 - 202)
Reply 6: Thank you for your question regarding the proportion of PR/SD/CR/PD across the four study arms. In response to your inquiry, we have conducted a thorough statistical reanalysis and have updated our manuscript accordingly. Our analyses, as indicated by the p-values, suggest that there is no significant difference in the proportion of PR/SD/CR/PR between the four arms of the study.
Changes in the text: We added "(p=0.131)" and "(p=0.435)" (see Page 6 line 215, line 217).

Comment 7: What is the difference between Figure 2A and Supplementary Figure 1. If the latter was KM curve after multivariate analysis, the manuscript should clearly state so. If not, a KM curve after multivariate analysis should be included (or clearly state why this cannot be done).
Reply 7: Thank you for value comments. Figure 2A presents the KM survival curves for the overall study population, illustrating the survival probabilities over time for each of the four maintenance therapy regimens (I+C+A, I+C, I+A, and I alone) in patients with advanced NSCLC (The time from initial treatment to disease progression). Supplementary Figure 1, by contrast, focuses specifically on the period beginning with the commencement of maintenance therapy, omitting the initial combined induction phase. It displays the Kaplan-Meier curves from the beginning of maintenance phase to disease progression, thus offering a more targeted analysis of this particular treatment segment across the four groups.

Furthermore, I appreciate the opportunity to discuss the analytical approach of our study further. To clarify, the Kaplan-Meier (KM) survival analysis used in our research serves to estimate survival probabilities over time without adjusting for other variables. This method suited our primary objective, which was to compare the survival outcomes of different maintenance therapy regimens for advanced non-small cell lung cancer (NSCLC) directly. We observed the survival impact of the regimens (I+C+A, I+C, I+A, and I alone) in a straightforward manner, which was the intended focus of our study.

On the other hand, a multivariate survival analysis, such as the Cox proportional hazards model, is crucial when the research objective includes assessing how multiple risk factors influence survival time. This type of analysis offers a more precise estimate of the independent effect of each factor on survival time, controlling for other variables. It is particularly necessary when exploring the relationship between survival time and a range of factors.

In the context of our study, the majority of baseline characteristics among participants in all 4 groups were similar. We also performed subgroup KM analysis in each baseline data. Given this homogeneity, and considering our focus was not on the interplay of multiple risk factors but rather on the direct comparison of treatment regimens, we opted for the KM analysis as our primary analytical tool. We believe this method sufficiently highlights the relative effectiveness of the treatment regimens without the confounding influence of other variables.

Therefore, while we acknowledge the value of multivariate analysis in the appropriate context, we maintain that our methodological choice is well-aligned with our study's aims. We provided a clear, direct assessment of the treatment regimens' survival outcomes, which would be the immediate concern for clinicians considering maintenance therapy options for patients.

Comment 8: (Line 214 - 218) Based on the data presented, I am not sure whether the claim of benefit of “I+A” over “I” can be made when stratified by PD-L1 level.
Reply 8: Thank you for your insightful query regarding the claim of superiority of the "I+A" treatment over "I" when stratified by PD-L1 level. In our analysis, there is a trend that the "I+A"

treatment group consistently showed improved outcomes compared to the "I" group across all levels of PD-L1 expression. These findings suggest a potential benefit of the combination therapy irrespective of PD-L1 status. However, we acknowledge the limitation imposed by the sample size of our study, which may affect the robustness of the stratified analysis. While our results are promising, we concur that larger, prospective studies are warranted to substantiate the benefits of "I+A" over "I" in the context of PD-L1 expression levels.

Comment 9: (Chemo-holiday regimen) This should not be part of the "result". Please consider including it in the discussion instead.
Reply 9: Thank you for your recommendation to include the chemo-holiday regimen in the Discussion section. I would like to provide some context for positioning this regimen within the Results section. The rationale for its placement was to emphasize the significance of this novel treatment approach directly derived from our study's findings. The regimen, supported by supplemental Figure 4, represents a pivotal aspect of our results, illustrating a practical application of our data.

We believe that the presentation of the chemo-holiday regimen alongside our findings helps to highlight its importance and the direct link to the empirical evidence we have provided. However, we understand the convention of discussing the implications of findings within the Discussion section. To address your concerns while maintaining the prominence of this concept, we had introduced the chemo-holiday regimen in brief within the Results section, and provided a more detailed exploration, including its broader implications and potential for future research, in the Discussion section. This approach ensures that the regimen is adequately highlighted as a significant outcome of our research, while also satisfying the traditional structure of a scientific manuscript.

Comment 10: Finally, please consider shortening the discussion session.
Reply 10: Thank you for your careful reading of our manuscript and for suggesting that we consider shortening the discussion section. We have reviewed the discussion carefully in light of your comment and believe that each part contributes significantly to the overall narrative and understanding of our study's findings. We have endeavored to provide a comprehensive analysis of our results in the context of the existing literature, to address the potential implications and limitations of our work, and to suggest avenues for future research. Given the c novelty of our findings, we feel that the current length is necessary to fully convey the relevance and potential impact of our study. However, we understand the importance of conciseness and have therefore scrutinized our discussion to ensure that it is as succinct as possible without omitting critical content. We have made some minor edits, but we have retained all sections of the discussion as each paragraph adds important context or interpretation that is integral to the reader's understanding of our research. We hope that upon reevaluation, you will find the discussion section to be comprehensive and necessary for the depth and clarity it provides to the study.

Reviewer C

Comment 1: Firstly, there were several major maintenance therapies, such as Chemo+ ICI (KEYNOTE 186), ICI+ ICI (9LA study, POSEIDON study), ICI + VEGF (IMPOWER 150), and ICI only (Pembro, NIVO et al). There was limited study support for using Chemo+ICI + VEGFR(Group 1) as maintenance therapy.

Reply 1: We gratefully appreciate for your valuable comment. Our study was a real-world retrospective study, and based on our inclusion and exclusion criterias, we concluded that there were 4 real-world maintenance therapies (ICIs+chemo+anti-VEGFR, ICIs+chemo, ICIs+ anti-VEGF and ICI only). In the LEAP-006 clinical trial, pembrolizumab plus pemetrexed with or without lenvatinib were used as maintenance regimen for advanced NSCLC. The final analysis of the research indicated that, compared to the combination of pembrolizumab with chemotherapy, the group treated with pembrolizumab plus lorlatinib and chemotherapy did not show an improvement in OS. Early interim analyses did not reveal statistically significant enhancements in PFS or in the ORR. In our stdy, for ICIs+chemo+ anti-angiogenesis maintenance regimen, we found that its efficacy was comparable to that of ICIs+chemo maintenance. So, when chemotherapy was used as maintenance, additional anti-angiogenesis did not contribute to better PFS. ICIs+chemo+ anti-angiogenesis maintenance regimen did not contribute to better survival. Our study findings are consistent with the results of LEAP-006, which also corroborates from another perspective that I+C+A maintenance regimen is not warranted for broader application in subsequent treatments.

Comment 2: Secondly, the authors didn't consider the differences in initial chemotherapy, which may also affect the effectiveness of chemotherapy.

Reply 1: Thank you for your insightful comments and suggestions. In our study, among the patients with lung adenocarcinoma (N=83), 73 were initially treated with pemetrexed plus platinum, and 10 received albumin-bound /liposomal paclitaxel plus platinum as their initial chemotherapy. For patients with squamous cell carcinoma of the lung (N=45), 17 were treated with gemcitabine plus platinum, and 28 received albumin-bound /liposomal paclitaxel plus platinum. In other types of tumors, 4 patients were initially treated with pemetrexed plus platinum, 4 with albumin-bound /liposomal paclitaxel plus platinum, and another 4 with gemcitabine plus platinum. Due to the limited number of patients, it was difficult to compare the impact of different initial chemotherapy regimens across the four groups. For the I+C+A and I+C treatment groups, the patients' initial chemotherapy regimen was almost exclusively pemetrexed plus platinum, preventing a comparison of the differences between initial chemotherapy regimens. However, this issue is a meaningful one that we will continue to monitor. We are conducting a prospective study to explore the impact of different maintenance strategies on patient prognosis, and we will compare the effects of different initial chemotherapy regimens on patient outcomes in subgroup analyses later on. The detailed feedback provided by the reviewers has been instrumental in enhancing the accuracy and depth of my research.

Comment 3: Thirdly, the authors should have identified the immunohistochemistry criteria of TPS in PD-L1, such as 22C3.

Reply: Thank you very much for your careful check and suggestions. In our article, the staining of tumor cells for PD-L1 is PD-L1 IHC 22C3 pharmDx, and we have added relevant

instructions in the revised manuscript.
Changes in the text: We added a statement as “PD-L1 expression on tumor cells was detected by PD-L1 IHC 22C3 pharmDx” (see page 7 line 232-233).

Reviewer D

Comment 1: Overall, the observations are impressive for developing optimal efficacy of the first-line chemotherapy for driver gene mutation-negative NSCLC and fit well within the scope of the journal, however, it would be better to add some more information and analysis to make the manuscript relevant for readers.
Reply 1: Thank you very much for your careful review and valuable comments regarding our manuscript. Based on your and other reviewers' comments, we have added more information to the article, and your comments have improved the depth and quality of our article.

Comment 2: Subjects are categorized into four groups, I+C+A, I+C, I, and I+A, however, a combined maintenance treatment with I+C+A is not a standard practical regimen in most of regions.
Reply 2: Thank you for the careful attention. Another reviewer raised a similar question. This is a real-world retrospective study. Indeed, in the real world, there are some physicians who use I+C+A as a maintenance treatment. Although the number is small, we have included these patients to further analyze the efficacy of this maintenance regimen. Our study results show that I+C+A is not the optimal maintenance strategy and is not worth promoting. When I+C is used as a maintenance regimen, the additional inclusion of anti-angiogenic drugs did not contribute to better survival benefit for patients. Furthermore, the incidence of toxic side effects may be higher due to the use of multiple drugs. The LEAP-006 prospective clinical trial is comparing the efficacy of the I+C+A (pembrolizumab plus pemetrexed with lenvatinib) with the I+C (pembrolizumab plus pemetrexed) maintenance regimen. Its results are consistent with ours and provide further evidence that I+C+A maintenance is not the optimal option for patients.

Comment 3: Subjects treated with anlotinib as an anti-angiogenesis agent were eligible for this study. This multi-kinase inhibitor is not approved outside China and does not have equivalent mechanisms of action with the more common anti-angiogenesis, bevacizumab.
Reply 3: Thank you for your comment concerning the inclusion of anlotinib as an anti-angiogenesis agent in our study. We acknowledge that anlotinib is currently approved for use only in China and that its mechanisms of action differ from those of more widely recognized anti-angiogenic agents such as bevacizumab.

The rationale for including anlotinib in our analysis is twofold. First, our study aims to reflect the full spectrum of real-world practices, which includes the application of region-specific treatments such as anlotinib in the management of advanced non-small cell lung cancer (NSCLC). Given the scope of our study, it is imperative to consider all available therapies that are actively being used in clinical settings, even if their use is geographically limited.

Second, despite the differences in mechanisms of action between anlotinib and bevacizumab, both drugs serve the broader therapeutic role of angiogenesis inhibition. Including both agents provides a more comprehensive understanding of the potential range of efficacy and safety profiles for anti-angiogenic strategies in NSCLC maintenance therapy.

We recognize that different anti-angiogenesis drugs in different regions limit the generality of our findings. The mechanisms of these anti-angiogenesis drugs differ slightly, and there have been no head-to-head trials to verify which anti-angiogenesis drugs work best. Therefore, we have taken care to explicitly state this limitation in our discussion section (see Page 10 line 352-353).

We believe that our findings contribute valuable insights into the comparative effectiveness of various maintenance strategies, including those not yet globally approved. Our results may inform future clinical decisions and serve as a preliminary basis for international clinical trials that could lead to wider acceptance of new treatment options.

Comment 4: Recently, it was reported that treatment with pembrolizumab in combination with lenvatinib, another multi-kinase inhibitor did not achieve a significant improvement in either OS or PFS as first-line treatment of adult patients with NSCLC without EGRF, ALK, or ROS1 alteration.
Reply 4: Pembrolizumab and lenvatinib have achieved remarkable results in clinical studies for gastric cancer, endometrial cancer, and melanoma, especially in liver cancer. However, in a phase Ib/II clinical study, the combination of pembrolizumab and lenvatinib as a subsequent treatment for advanced NSCLC showed an objective response rate (ORR) of 33.3% and a median progression-free survival (PFS) of 5.9 months. This combination did not significantly improve the survival of patients with advanced non-small cell lung cancer. However, another IB clinical study showed that in NSCLC patients received sintilimab and anlotinib as first line treatment, the ORR was 72.7% and the disease control rate (DCR) was100%. Median PFS was 15 months (PMID: 33524601). Currently, there is a lack of phase III study data on the frontline treatment of advanced NSCLC with the combination of immune checkpoint inhibitors. We believe that ICIs combined with anti-angiogenesis drugs still has great prospects as first-line therapy.

Recent literature is increasingly focused on developing chemo-free strategies to optimize patient outcomes while minimizing the adverse effects associated with chemotherapy. In the context of advanced non-small cell lung cancer, the integration of ICIs with anti-angiogenic drugs as primary treatment is still being explored. The efficacy of varying drug combinations is likely to influence patient prognoses differently. Our study acknowledges the beneficial role of chemotherapy during the initial phase of treatment. Subsequently, we observed that a maintenance therapy combining ICIs with anti-angiogenic agents yielded results comparable to those of the combined immunotherapy and chemotherapy regimen.

Comment 5: From there, it would be better to show all regimens and subject numbers of each

regimen, especially for anti-angiogenesis agents. If possible, separate analysis for I+bevacitumab and I+anlotinib groups would be preferred.

Reply 5: Thank you for your insightful query regarding the detailed presentation of treatment regimens, particularly concerning the use of anti-angiogenesis agents. We understand the interest in a separate analysis for the I+bevacizumab and I+anlotinib groups to potentially discern differential impacts on patient outcomes. In our study, within the I+A (immunotherapy plus anti-angiogenesis) maintenance treatment group, 31 patients were treated with anlotinib due to its broader indications, which include squamous cell carcinoma, adenocarcinoma, and small cell lung cancer, among others. Because bevacizumab is only indicated for lung adenocarcinoma, only 7 patients received bevacizumab.

Given this disparity in patient numbers between the anlotinib and bevacizumab subgroups, performing a comparative analysis to effectively evaluate the difference in maintenance outcomes posed by these distinct anti-angiogenesis agents is challenging. The limited sample size, particularly for the I+bevacizumab group, restricts the statistical power needed to draw meaningful conclusions.

We have acknowledged this limitation within our manuscript, suggesting that the question of comparative effectiveness between different anti-angiogenesis drugs in maintenance therapy is best addressed through a larger, prospective study designed with this specific aim in mind.

Comment 6: The distribution of sites of metastases is not equivalent in each group. In group 4, liver and brain metastases were relatively lower than in other groups. Since these organs are critical for survival, small numbers of metastases in these sites would provide better outcomes for group 4.

Reply 6: Thank you for your insightful comments regarding the distribution of metastatic sites in each group. We recognize the importance of the liver and brain metastases for patient survival outcomes, as you have noted. In our analysis, although group 4 does exhibit a lower incidence of liver and brain metastases, it is important to consider that this group has a higher proportion of patients with stage IV cancer, accounting for 63.2% of its composition. This advanced stage of disease inherently carries a worse prognosis, which may offset the potential benefit of having fewer critical metastases. We believe that this consideration can partly explain the survival outcomes in group 4.