Peer Review File
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Reviewer A

1) First, the title needs to indicate efficacy and safety outcomes and the accurate clinical research design of this study, i.e., a retrospective cohort study.

Reply 1:We have modified our title as advised（see Page 1,line4）.

Changes in the text: Etoposide soft capsule combined with anlotinib in the third-line treatment of advanced non-small cell lung cancer: a retrospective cohort study

2) Second, the abstract is not adequate and needs some revisions. The background did describe the knowledge gap and what the potential clinical significance of this research focus is. The methods need to describe the inclusion of subjects, the assessment of baseline factors, follow up procedures, and measures of efficacy and safety outcomes. The results need to summarize the baseline clinical characteristics of the study sample. The conclusion needs to be tone down since there is no a control group and the sentence “a certain clinical effect” is vague and unclear.

Reply 2:We have modified our text as advised（see Page 1-2,line36-50）.

Changes in the text: **Methods:** A retrospective study was conducted on 46 patients with advanced NSCLC who had failed second-line treatment. Progression-free survival (PFS) of advanced NSCLC patients served as an endpoint. Kaplan-Meier survival curves were applied to evaluate the short-term efficacy of anlotinib treatment in advanced NSCLC patients.

Results: Among 46 third-line NSCLC patients, none had complete remission (CR), 9 had partial remission (PR), 29 had stable disease (SD), and 8 had progressive disease (PD). The objective response rate (ORR) was 19.57%, the disease control rate (DCR) was 82.61%, the median progression-free survival (mPFS) was 6.3 months, and the median overall survival (mOS) was 10.1 months. Common adverse reactions included fatigue, hypertension, nausea, stomatitis, leukopenia, hand-foot syndrome, abnormal liver function, proteinuria, hemoptysis, and hypothyroidism, among others. The incidence of grade 3 adverse reactions was 8.9%, and there were no grade 4 adverse reactions.

Conclusions: Etoposide soft capsule combined with anlotinib demonstrated a marked effect on the third-line treatment of advanced NSCLC patients,and is well tolerated.

3) Third, the introduction of the main text needs to review what has been known on the third-line treatment of advanced non-small cell lung cancer including their efficacy

and safety data, please also analyze the limitations of prior studies, and explain why etoposide soft capsule combined with anlotinib is potentially effective and safe and why this focus deserves to be studied.

Reply 3:We have modified our text as advised（see Page 3,line83-101）.

Changes in the text: Shao et al. have shown that the median progression free survival (PFS) values in the single chemotharpy agent, EGFR-TKIs and doublet chemotherapy groups were 2.30, 3.17 and 2.37 months. The rates of stage III-IV toxicities were 33.3%, 18.2% and 68.8%(3).With the emergence of more low-toxic chemotherapy and small-molecule targeted drugs, more patients still have the opportunity to receive third-line treatment after first-line and second-line treatment, and third-line treatment is superior to the best supportive treatment, which has been confirmed by a number of studies [4-6]. We need more efficient and less toxic solutions.

Anlotinib was approved by the Chinese Food and Drug Authority (FDA) in 2018 for the third-line monotherapy of advanced NSCLC (7). In the ALTER0303 study, the median progression-free survival (mPFS) in the anlotinib group was 5.4 months, the median overall survival (mOS) was 9.6 months, the objective response rate (ORR) was 9.2%, and the disease control rate (DCR) was 81.0% (8). Zhong et al also showed that anlotinib demonstrated a marked effect on the clinical treatment of advanced NSCLC patients; it could effectively prolong the PFS of patients, was well tolerated and safe(9).

Studies have shown that the ORR of anlotinib combined with chemotherapeutic drugs can reach 27%, and the DCR can reach 78% (10), but there are few related reports. Etoposide soft capsules are commonly used oral chemotherapeutic drugs in clinical practice. This is a clinically accessible combination of etoposide and anlotinib.

4）Fourth, in the methodology of the main text, please describe the clinical research design, sample size estimation, and details of follow up. In statistics, please ensure $P<0.05$ is two-sided.

Reply 4:We have described the relevant contents in the section of clinical data（see Page 3-5,line108-160）.We also modified our text as advised（see Page 5,line166-167）.

Changes in the text: All statistical tests were two sided, and $P<0.05$ was considered to be statistically significant.

5） Finally, please review and cite several related papers: 1. Zhu Q, Ni R, Guan X. Cost-effectiveness analysis of anlotinib as a third-line or further treatment for advanced non-small cell lung cancer in China. Transl Lung Cancer Res 2023;12(8):1782-1789. doi: 10.21037/tlcr-23-456. 2. Zhao Y, Wang Q, Zhang L, Shi J, Wang Z, Cheng Y, He J, Shi Y, Chen W, Luo Y, Wu L, Wang X, Nan K,

Jin F, Dong J, Li B, Yamaguchi F, Breadner D, Nagano T, Tanaka F, Husain H, Li K, Han B. The efficacy of anlotinib as third-line treatment for non-small cell lung cancer by EGFR mutation status: a subgroup analysis of the ALTER0303 randomized phase 3 study. Transl Lung Cancer Res 2022;11(5):776-785. doi: 10.21037/tlcr-22-320. 3. Diebels I, Van Schil PEY. Diagnosis and treatment of non-small cell lung cancer: current advances and challenges. J Thorac Dis 2022;14(6):1753-1757. doi: 10.21037/jtd-22-364.

Reply 5:We have modified our text as advised（see Page 7,line251-261）.

Changes in the text: It is commonly believed the clinical benefit from chemotherapy may be reduced substantially after EGFR TKI treatment (23). But in the ALTER0302 study, patients with EGFR mutation who had disease progression after EGFR TKI treatment and chemotherapy, anlotinib achieved a PFS of 5.6 months and an OS of 10.7 months(24).In our study, it was also shown that patients with EGFR mutation who had disease progression after EGFR TKI treatment , our combination regimen achieved a PFS of 7.0 months. Monitoring ctDNA is important in the treatment of lung cancer, It can guide drug use and help analyze the mechanism of drug resistance(25).But this study is a retrospective study, the genetic test results of these patients with EGFR mutations are not complete, and further studies cannot be conducted to explore which patients are more likely to benefit from anlotinib.

Reviewer B

The paper titled “Etoposide soft capsule combined with anlotinib in the third-line treatment of advanced non-small cell lung cancer: a retrospective study” is interesting. Etoposide soft capsule combined with anlotinib has a certain clinical effect in the third-line treatment of advanced NSCLC, and is well tolerated. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In order to further verify the role of etoposide soft capsule combined with anlotinib, it is necessary to further accumulate clinical cases and conduct larger sample, multi-center, randomized, and controlled clinical trials.

Reply 1:We have modified our text as advised（see Page 9,line283-289）.

Changes in the text: The present study had limitations. The study did not include front-line treatment of immunotherapy patients.The efficacy of this combined regimen after immunotherapy could not be evaluated.And the sample size was small in subgroups, and it was difficult to draw firm conclusions in specific populations. In order to further

verify the role of etoposide soft capsule combined with anlotinib, it is necessary to further accumulate clinical cases and conduct larger sample, multi-center, randomized, and controlled clinical trials.

2) What are the advantages of combination therapy? It is recommended to add relevant comparative analysis.

Reply 2: We have modified our text as advised（see Page 3,line86-101）.

Changes in the text: With the emergence of more low-toxic chemotherapy and small-molecule targeted drugs, more patients still have the opportunity to receive third-line treatment after first-line and second-line treatment, and third-line treatment is superior to the best supportive treatment, which has been confirmed by a number of studies [4-6]. We need more efficient and less toxic solutions.Anlotinib was approved by the Chinese Food and Drug Authority (FDA) in 2018 for the third-line monotherapy of advanced NSCLC (7). In the ALTER0303 study, the median progression-free survival (mPFS) in the anlotinib group was 5.4 months, the median overall survival (mOS) was 9.6 months, the objective response rate (ORR) was 9.2%, and the disease control rate (DCR) was 81.0% (8). Zhong et al also showed that anlotinib demonstrated a marked effect on the clinical treatment of advanced NSCLC patients; it could effectively prolong the PFS of patients, was well tolerated and safe(9).Studies have shown that the ORR of anlotinib combined with chemotherapeutic drugs can reach 27%, and the DCR can reach 78% (10), but there are few related reports. Etoposide soft capsules are commonly used oral chemotherapeutic drugs in clinical practice. This is a clinically accessible combination of etoposide and anlotinib.

3) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 3:We have modified our text as advised（see Page 3,line83-90）.

Changes in the text: Shao et al. have shown that the median progression free survival (PFS) values in the single chemotharpy agent, EGFR-TKIs and doublet chemotherapy groups were 2.30, 3.17 and 2.37 months. The rates of stage III-IV toxicities were 33.3%, 18.2% and 68.8%(3).With the emergence of more low-toxic chemotherapy and small-molecule targeted drugs, more patients still have the opportunity to receive third-line treatment after first-line and second-line treatment, and third-line treatment is superior to the best supportive treatment, which has been confirmed by a number of studies [4-6]. We need more efficient and less toxic solutions

4) What is the impact of this study on the further treatment and prognosis of NSCLC? What is the author's next research plan? It is recommended to include relevant content in the discussion.

Reply 4:We have modified our text as advised（see Page 9,line286-289）.
Changes in the text:In order to further verify the role of etoposide soft capsule combined with anlotinib, it is necessary to further accumulate clinical cases and conduct larger sample, multi-center, randomized, and controlled clinical trials.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Efficacy and safety of anlotinib in patients with advanced non-small cell lung cancer, J Thorac Dis, PMID: 33209434”. It is recommended to quote the article.

Reply 5:We have modified our text as advised（see Page 3,line95-97）.
Changes in the text: Zhong et al also showed that anlotinib demonstrated a marked effect on the clinical treatment of advanced NSCLC patients; it could effectively prolong the PFS of patients, was well tolerated and safe(9).

6) This study is a retrospective analysis, which is likely to cause some deviations in the results. It needs to be further confirmed by multi-center clinical trials.

Reply 6:We have modified our text as advised（see Page 9,line295-298）.
Changes in the text:However, given that the sample size of this study is small and that it is a retrospective study, a prospective study with a larger sample， multi-center, randomized, and controlled clinical trials can be conducted to further verify the above conclusions.

7) With the discovery of new drug targets and the continuous emergence of new combination treatment options, what breakthroughs will there be in the treatment of NSCLC in the future? What inspiration can this study provide? It is recommended to add relevant content to the discussion.

Reply7:We have modified our text as advised（see Page 9,line283-285）.
Changes in the text:The study did not include front-line treatment of immunotherapy patients.The efficacy of this combined regimen after immunotherapy could not be evaluated.