Peer Review File

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Reviewer A

It could be useful in the abstract to add the pharmaceutical class of Anlotinib as it is not specified.

Reply: Thanks for your advice, and we have added the pharmaceutical class of Anlotinib in the abstract.
Changes in the text: We have modified our text as advised (see Page 2, line 15-16).

Reviewer B

The authors Li et al here present a single arm phase 2 study of Osimertinib along with anlotinib, an angiogenesis targeting multi-target TKI as first line treatment in EGFR mutant NSCLC.
The authors and the study team should be commended for the efforts to conduct this study, especially including patients with ECOG 2 PS which is rarely done in first line trials.
The combination therapy was futile due to excessive toxicities and had to be stopped after 11 patients were enrolled. This still represents an important data that needs to be disseminated. However, several issues need to be addressed and clarified before this is publication ready.

Reply: Thank you for your feedback. We are submitting a revised manuscript to address these concerns. Detailed point-by-point responses to these concerns are provided hereinunder.

Major:

1. To my knowledge, this is the first study to test the combination of EGFRi and VEGF TKI's in EGFR mutant NSCLC. While the theoretical rationale for the combination is sound, it is to be noted that the previous positive studies of VEGF targeting with Bevacizumab and ramicirumab are monoclonal antibodies against VEGF and VEGFR respectively. These drugs have no effect on other targets (FGFR, PDGFR etc) like anlotinib. This has to be reconciled in the paper and discussed.
Reply: The main aim of this study was to explore better combination treatment strategies for postponing drug resistance of the third-generation EGFR-TKIs in untreated EGFR-mutant NSCLC combined with antiangiogenic drugs. The previous positive studies of EGFR TKIs combined with bevacizumab and ramicirumab were important bases of this study and have been discussed in the paper. Anlotinib is a multitarget antiangiogenic TKI by oral administration, which is more convenient for patients to use. Meanwhile, concurrent use of anlotinib have already been reported to overcome acquired resistance to EGFR-TKIs in advanced *EGFR*-mutant NSCLC patients (see Page 5, line 24-34). Based on these, anlotinb was chosen as the combine regimen.

Changes in the text: None.

2. Secondly, anlotinib being a promiscuous multi-target TKI (VEGFR, FGFR, PDGFR etc) it is expected to have significant potential for on target toxicities. While the ALTER-0303 used 12 mg daily as the dose (Days 1-14 of 21-day cycle), the authors don't provide a good rationale for why they chose the 12 mg every other day in combination with full dose Osimertinib, as opposed to 6 mg daily? Was there PK data available to suggest if this dosing strategy was better. Was PK done as a part of the study? Doing this dosing strategies will lead to lot of peaks and troughs of drug concentration and may potentially lead to more toxicities.

Reply: In clinical practice of our cancer center, some patients with mutated EGFR who received a third-generation EGFR-TKI as initial therapy then simultaneously received anlotinib when disease progression occurred. We observed that most of them who received standard-dose anlotinib treatment required dose adjustments due to adverse events, and we found it was well tolerated when given at the dose of 12 mg every other day. It could not be given 6 mg daily because its specification was 12mg per tablet which was not suitable to cut into two pieces. PK was not a part of the study, so there was no PK data available to suggest if this dosing strategy was better.
Changes in the text: None.

3. More details on the statistical methodologies are needed. How was the sample size of 35 calculated? What were the underlying assumptions?

Reply: The reported ORRs were 80% of osimertinib alone, and 73.8% of aumolertinib alone. The average ORR was 76.9%. 31 events were deemed necessary to detect an ORR of 95.1% of the third generation EGFR-TKIs plus anlotinib, with a 2-sided significance level of 0.05 and a power of 0.8. The target sample size was set at 35, allowing for dropouts.
Changes in the text: We have modified our text as advised (see Page 6, line 23-27).

4. It is not clear why was a phase 1b study to study this combination with escalating dose? Why did the authors not use any statistical models to monitor excessive toxicity?

Reply: As mentioned in Reply of Question 2, in clinical practice of our cancer center, some patients with mutated EGFR who received a third-generation EGFR-TKI as initial therapy then simultaneously received anlotinib when disease progression occurred. We found it was well tolerated when anlotinib was given at the dose of 12 mg every other day. Therefore, we speculated that it would be equally safe when administrated in the same way as an initial therapy. But the outcome turned out to be really beyond expectation.
Changes in the text: None.

5. Why did the authors not incorporate early safety endpoints or staggering of patients between enrollment to avoid excess toxicities?

Reply: As mentioned in Reply of Question 2, In clinical practice of our cancer center, some patients with mutated EGFR who received a third-generation EGFR-TKI as initial therapy then simultaneously received anlotinib when disease progression occurred. We found it was well tolerated when anlotinib was given at the dose of 12 mg every other day. Therefore, we speculated that it would be equally safe when administrated in the same way as an initial therapy. But the outcome turned out to be really beyond expectation.
Changes in the text: None.

6. The secondary endpoints of PFS and OS should be presented in the results, even though they may not be much relevant due to the effective sample size.

Reply: Neither median PFS nor OS was reached at the time of data cutoff. (see Page 8, line 24-25)
Changes in the text: None.

7. Although the ORR seen with combination in this study is significantly lower than expected (even with a small sample size), the authors should discuss that the ORR with single agent anlotinib was at best 10% in the previous randomized studies, although these studies were in the later lines of treatment.

Reply: The aim of the study was to explore whether combined use of anlotinib and the third-generation EGFR-TKIs would improve the efficiency of the monotherapy of the third-generation EGFR-TKIs as first-line treatment. The reported ORRs of single agent anlotinib were in the later lines of treatment. Besides, the status of driver alterations of enrolled subjects of these studies were quite different from our study. Therefore, we think it's not necessary to discuss the ORR with single agent anlotinib in the article.
Changes in the text: None.

8. Why did 4 patients did not get TKI after being taken off from the study? IS that because they continued the study drug combo, please clarify.

Reply: Yes, 4 patients continued the study drug combo.
Changes in the text: We have modified our text as advised (see Page 9, line 20-21).

Minor:

1. Since this drug was developed in China, one would like to know how the study was funded?

Reply: The study had no funding. Subjects participated in this study voluntarily.
Changes in the text: None.

2. Page 6 line 1, the nine patients are "per-protocol" and not "intention to treat" population (which is N=11).

Reply: Thanks for your advice, and we have changed "intention to treat" to "per-protocol".

Changes in the text: We have modified our text as advised (see Page 7, line 34).

Reviewer C

This study evaluated the combination of osimertinib and anlotinib, a VEGFR2 inhibitor, in patients with untreated EGFR-mutated NSCLC. The trial was unfortunately terminated due to trAE in more than half of the patients. There are several problems with this trial

The trial was terminated due to 5/9 serious AEs. However, the criteria for termination is unclear. Was this trial terminated based on the definitions set before the trial?

Reply: In clinical practice of our cancer center, some patients with mutated EGFR who received a third-generation EGFR-TKI as initial therapy then simultaneously received anlotinib when disease progression occurred. We found it was well tolerated when anlotinib was given at the dose of 12 mg every other day. Therefore, we speculated that it would be equally safe when administrated in the same way as an initial therapy. But the outcome turned out to be really beyond expectation. We did not set specific definitions when to terminate the trial before the study begun. But the incidence of serious AEs of the combined regimen was quite high compared with the monotherapy, and some occurred shortly after administration. Besides the AEs were likely related to the combined regimen. Thus, we made the decision to terminate the trial.
Changes in the text: None.

The number of evaluable cases is too small at 9. Nothing can be drawn from this number of cases.

Reply: As discussed in the article, we also believed it was too early to compare the efficacy of the combined regimen with the monotherapy due to the limitation of the number of evaluable cases. But combining third-generation EGFR-TKIs with anlotinib as the initial treatment might significantly increase toxicity in *EGFR*-mutant patients with advanced NSCLC. The aim of this article was to warn oncologists intending to use such combined therapy to evaluate comprehensively and choose carefully before treatment.
Changes in the text: None.

Akamatsu et al. conducted a phase Ib trial of osimertinib in combination with ramcirumab and found good anti-tumor efficacy and in one case only, G3 appetite loss. The present study contradicts this result. Although there is a difference between second-line and first-line, the patient's condition may be better in this trial conducted in the first-line setting. Is the higher toxicity due to anlotinib rather than the combination?

Reply: As mentioned in the article, one patient who received anlotinib and aumolertinib quitted the trial due to grade 3 interstitial pneumonia. But the interstitial pneumonia of this patient aggravated again after starting subsequent aumolertinib treatment alone. Thus, we thought the higher toxicity was due to the combined regimen rather than anlotinb.

Changes in the text: None.

Reviewer D

The authors report the results of a small phase II trial investigating combination therapy of anlotinib with third-generation EGFR-TKI. Because of toxicity, enrollment was discontinued after 11 of 35 patients were treated. The finding of inappropriate toxicity of the combination of anlotinib and osimertinib/aumolertinib is important. However, the small size of this single-centre study are limiting the significance of the results and should be clearly indicated, e.g. in the title.

Title:
- I recommend to replace „phase II "by „pilot"

Reply: Thanks for your advice, and we have changed "phase II" to "pilot".
Changes in the text: We have modified our text as advised (see Page 1, line 2).

Introduction
- P. 3, ll. 35-47, p. 4, ll. 1-10: The benefit-risk ratio of combined treatment of EGFR-TKI and VEGF inhibitors is not correctly presented. Of course, combined treatment comes along with additional safety issues.

Reply: Thanks for your comments, and we are learning to present the benefit-risk ratio scientifically in our future work.
Changes in the text: None.

- P. 4, l. 7 „no unexpected serious adverse events were observed. " This does not mean that the combination is safe. Most SAEs are expected. This sentence is misleading and should be deleted.

Reply: Thanks for your advice, and we have deleted "no unexpected serious adverse events were observed".
Changes in the text: We have modified our text as advised (see Page 5, line 4).

- The efficacy of combined treatments in regards of overall survival should be mentioned as well.

Reply: Thanks for your advice, and we have added the data of overall survival of the combined treatments.
Changes in the text: We have modified our text as advised (see Page 4, line 28-30 and 32-34; Page 5, line 4-7 and 10-11).

Methods
- Where has the trial been registered?

Reply: The trial has been registered in Chinese Clinical Trial Registry (see Page 2, line 15).
Changes in the text: None.

- P. 5, Patients: „The planned enrollment was 35 patients, calculated using the Objective 7 Performance Criteria for single-arm clinical trials by SPSS. “ How was the calculation done in detail? A patient number of 35 appears very low.

Reply: The reported ORRs were 80% of osimertinib alone, and 73.8% of aumolertinib alone. The average ORR was 76.9%. 31 events were deemed necessary to detect an ORR of 95.1% of the third generation EGFR-TKIs plus anlotinib, with a 2-sided significance level of 0.05 and a power of 0.8. The target sample size was set at 35, allowing for dropouts.
Changes in the text: We have modified our text as advised (see Page 6, line 23-27).

- Study assessments: When and how often were the safety assessments performed? By whom were the CT scans evaluated? Were the examiners informed about the study treatment? What about vital parameters, safety lab, physical examination etc.? The study protocol should be added as a supplement.

Reply: During treatment, the regular safety assessments were performed every 6 weeks and immediate safety assessments would be performed whenever subjects showed signs of AEs provided by subjects themselves or noticed by our investigators. The CT scans were evaluated by both investigators and Imaging specialists. The examiners were informed about the study treatment. According to the Data Sharing Statement, we are pleased to share data collected for our study including vital parameters, safety lab, physical examination etc. and the study protocol.
Changes in the text: None.

- Statistical analysis: The authors describe an intention-to-treat analysis. However, patients lost to follow-up were not included in the analysis. In an intention-to-treat analysis, all patients are counted in the group they were originally assigned to, even if the data are not complete.

Reply: Thanks for your advice, and we have changed “intention to treat” to “per-protocol”.
Changes in the text: We have modified our text as advised (see Page 7, line 34).

Results
- AEs of grade 3 or higher are have been observed under combined treatment regimens in the majority of patients (54 – 99% of patients, see Hafner et al. 2021, doi: 10.1038/s41392-021-00813-y).

Reply: Thanks for your comments. We learned more about the fact that AEs of grade 3 or higher had occurred under combined treatment regimens of anti-VEGF plus EGFR-TKI as first-line treatment in the majority of EGFR-mutant NSCLC patients from this article.
Changes in the text: None.

Discussion

- P. 7, l. 19: „However, adding anlotinib to the 20 third-generation EGFR-TKIs significantly increased the incidence of trAEs.“ E.g., in the WJOG9717L trial by Kenmotsu et al., AEs of grade 3 or higher affected 48% of patients under osimertinib.

Reply: Thanks for your comments. We have modified this sentence to precisely clarify the message we delivered.
Changes in the text: We have modified our text as advised (see Page 9, line 29-30).

- Further limitations of the study should be discussed (single arm, single center, unblinded etc.)

Reply: Thanks for your advice, and we have discussed the limitations of the study including single arm, single center and unblinded etc.
Changes in the text: We have modified our text as advised (see Page 10, line 23-25).

Figure 1

- The figure legend should indicate the time of treatment and the time of assessment of the endpoints.

Reply: The time of treatment of each subject were presented in Table 1. The time of assessment of the endpoints from has been added in Table 1.
Changes in the text: None.