Peer Review File

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Reviewer A

The manuscript entitled "Triple-targeted therapy of dabrafenib, trametinib, and osimertinib for the treatment of the acquired BRAF V600E…" investigates the efficacy and safety of a triple-targeted therapy in patients with BRAF and EGFR mutations. This is a retrospective multi-center review of 13 cases. The manuscript is well written and the study well conducted. This is a topic of interest as adjusted treatment strategies after osimertinib progression are currently lacking and because combined treatment strategies to overcome resistance mechanisms are not commonly investigated and offered to patients.

Comment 1: It would be helpful to include the precise sequence of treatments received by the 13 patients. Table 1 states that the triple treatment was given with 1-4 prior lines but we do not know what are the other treatments received and more importantly the exact timing between osimertinib alone and the triple treatment.
Reply 1: Thank you for the helpful suggestion. We have provided the treatment sequence information in revised Figure 1A.
Changes in the text: Revised Figure 1A.

Comment 2: In Fig2D the doses of each agent should be indicated. At 1 micromolar point are all the three drugs at the same dose?
Reply 2: Thank you for the important question. In this study, we employed normalized concentrations for different drugs based on findings from our previous research (Wang et al., 2023), given their varying pharmacological characteristics. Consequently, we standardized their respective concentrations to 1 when presenting our drug test results. We regret that the actual concentration of each compound was not provided in the previous version of the manuscript, as this omission may have caused misunderstanding. The details of the compounds utilized in this study are presented in supplementary Table 1, and this information has been included in the revised manuscript.

Supplymentary Table 1. Details of drug compounds used in this study and their normalized concentrations

	Supplier	Cat. No.	The actual concentration when the normalized concentration is 1	Maximun concentration for drug test
Osimertinib	MCE	HY-15772	2 μM	8 μM
Dabrafenib	MCE	HY-14660	2 μM	8 μM
Trametinib	MCE	HY-10999	0.2 μM	0.8 μM
Vemurafenib	MCE	HY-12057	2 μM	8 μM
Encorafenib	MCE	HY-15605	2 μM	8 μM
Pemetrexed	MCE	HY-10820	75 μM	300 μM
Carboplatin	MCE	HY-17393	12.5 μM	50 μM

Reference:

WANG, H. M., ZHANG, C. Y., PENG, K. C., CHEN, Z. X., SU, J. W., LI, Y. F., LI, W. F., GAO, Q. Y., ZHANG, S. L., CHEN, Y. Q., ZHOU, Q., XU, C., XU, C. R., WANG, Z., SU, J., YAN, H. H., ZHANG, X. C., CHEN, H. J., WU, Y. L. & YANG, J. J. 2023. Using patient-derived organoids to predict locally advanced or metastatic lung cancer tumor response: A real-world study. Cell Rep Med, 4, 100911.

Changes in the text: Figure 2D, Supplementary table 1, Methods

Comment 3: This may not be possible to perform but having osimertinib alone and BRAF inh alone would have been helpful to prove that triple treatment is more potent than single agents.

Reply 3: Thank you for the constructive comment. While it would indeed be helpful to have osimertinib alone and BRAF inhibition alone as controls to prove the superior effect of the triple treatment, this was a retrospective real-world study and such information was not collected. It is noteworthy, however, that patient No. 13 initially received dabrafenib and trametinib after experiencing progression on EGFR-TKI therapy upon the emergence of a BRAF V600E mutation. Although a partial response was initially achieved with the dual therapy, the disease progressed after 3 months. Subsequently, triple-targeted therapy was started, resulting in a partial response (PR) after two months. This case reflects to some extent the efficacy of the triple treatment.

Changes in the text: No change.

Comment 4: In Fig4A it would be easier to read the panel with the patients positioned in the same order from left to right between pre and post.
Reply 4: We have revised Figure 4A accordingly.
Changes in the text: Figure 4A.

Reviewer B

This study is the first to show triple-targeted therapy against acquired BRAF V600E mutation after progression on EGFR-tyrosine kinase inhibitors in advanced EGFR-mutated non-small cell lung cancer patients. The authors studied the combination of three small molecules that target the EGFR-BRAF-MEK signaling pathways. This triple combination treatment demonstrated a robust clinical response, a manageable safety profile, and a promising PFS of 13.5 months. I would recommend the manuscript for publication in "Translational Lung Cancer Research" after minor revisions.

Comment 1: Despite debrafenib's 8-10 h elimination half-life and 95% oral bioavailability, why was debrafenib dosed twice daily (150mg)?
Reply 1: Based on pharmacokinetics, tissue pharmacodynamics, FDG-PET pharmacodynamics, and dose-response relationships, a 150 mg BID dose achieves a pERK target inhibition rate of over 80%. Increasing the dose or frequency does not improve this inhibition rate. In dose exploration up to 300 mg BID, dabrafenib did not reach the maximum tolerated dose (MTD), and doses of ≥35 mg BID have shown antitumor activity (Reference: Dose Selection, Pharmacokinetics, and Pharmacodynamics of BRAF Inhibitor Dabrafenib (GSK2118436)).
In addition to PK explanations, the dose selection also considers clinical efficacy and toxicity. We used the standard dose from the drug label. However, the optimal dose for the triple-target combination therapy still requires further clinical trial exploration.
Changes in the text: No change.

Comment 2: For PDO models, IC50 values should be provided and potency discussed to determine which combination of targeted therapies provides the best efficacy vs safety.
Reply 2: The IC50 values for different drug combinations in PDO models are provided in the table below. The data indicate that the osimertinib + dabrafenib + trametinib combination has the lowest IC50 value. Combined with the inhibition rate shown in Figure 2E, this triple combination demonstrates the best inhibitory effect.

Drug Combination	IC50

Osimertinib + Vemurafenib	0.724
Pemetrexed disodium + Carboplatin	2.721
Osimertinib + Dabrafenib + Trametinib	0.3838
Osimertinib + Encorafenib + Cetuximab	0.86832

Changes in the text: No change.

Comment 3: Authors have to include all missing all raw data figures (Kaplan -Meier survival curves).

Reply 3: We have added a Kaplan-Meier survival curve for progression-free survival (PFS) as Figure 1B. The numbering of subsequent figures has been adjusted accordingly.

Changes in the text: Figure 1

Reviewer C

The authors demonstrate in a retrospective Phase 1 clinical trial that the combination of dabrafenib, trametinib, and osimertinib in the EGFR TK1I which acquired BRAFV600E. The authors demonstrate the therapeutic efficacy of this combination therapy, and I was remarkably impressed by the results on the CT scan. Congratulations to the Authors! They extended this data to generating PDO and RNA seq which recapitulated the biology of the NSCLC studied.

Comment 1: The adverse effects of diarrhoea at 46% is quite high. It is possible that the authors could titrate a sub IC50 of the combo and see if the diarrhoea is reduced in further studies or actively treat for diarrhoea by rehydration before and during the studies

Reply 1: Thank you for your insightful comment. As this was a retrospective study, we were unable to adjust doses during the treatment period. However, we acknowledge the high incidence of diarrhea and agree that titrating a sub-IC50 of the combination therapy could potentially reduce this adverse effect. We plan to investigate this further in future prospective studies. Additionally, we will consider active management strategies for diarrhea, such as rehydration, before and during the studies to mitigate this side effect.

Changes in the text: No change.

Comment 2: The ethnicity of these patients was not listed. This must be included before the paper is accepted.

I recommend minor revision before acceptance to include the ethnic distribution as this is an important factor missed.

Reply 2: Thank you for pointing out this important factor. All included patients were Chinese. We will include this information in the revised manuscript to ensure the ethnic distribution is clearly stated.

Changes in the text: Abstract, Methods