Peer Review File

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Reviewer A

This is a study protocol paper entitled "Clinical advances of EGFR-TKIs combination therapy in EGFR-mutated non-small cell lung cancer (NSCLC): a Narrative Review". In this review paper, the authors described an extensive review on EGFR-TKIs combination study. This is a very important topic that contributed to literature, however, some points should be corrected before acceptance of this paper.

line 40-41: "Although sometimes there are some serious adverse events that lead to trial termination" appears to be a repetition of the sentence in Key Content and Findings. Please erase either sentence.
Reply: Thanks for your valuable comments. After careful consideration, we decided to remove the sentence in Key Content and Findings (see Page 2, line 35).

line 55: "Several NSCLC patients" may be a weird phrase. Please consider more appropriate wording.
Reply: Thanks for your valuable comments. We are sorry for our inappropriate expression. We have changed "several NSCLC patients" to " most NSCLC patients" (see Page 4, line 58).
Changes in the text: In addition, with in-depth research on the oncogenesis and progression of NSCLC, most NSCLC patients have been detected with mutations of the epidermal growth factor receptor (EGFR).

line 81-82: Reference 19 was published in 2010, which may be outdated. The authors should cite several, more recent references.
Reply: Thanks for your valuable comments. We have updated references to this section for better information (see Page 5, line 85 and Ref 20-21). By the way, we also removed outdated references elsewhere and cited more recent references, please see the revised manuscript.
Changes in the text:
[20] Leonetti, A.; Sharma, S.; Minari, R.; Perego, P.; Tiseo, M., Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *British Journal of Cancer* 2019, 121 (9), 1-13.
[21] Piper-Vallillo, A. J.; Sequist, L. V.; Piotrowska, Z., Emerging Treatment Paradigms for EGFR-Mutant Lung Cancers Progressing on Osimertinib: A Review. *Journal of Clinical Oncology* 2020, 38 (25), JCO.19.03123.

line 89: The authors should cite, if any, appropriate references in regard to anti-cancer efficacy through synergistic effects?

Reply: Thanks for your valuable comments. We have added references to this section for better information (see Page 5, line 93 and Ref 27-28).
Changes in the text:
[27]Liu, Z.; Gao, W., Synergistic effects of Bcl-2 inhibitors with AZD9291 on overcoming the acquired resistance of AZD9291 in H1975 cells. *Archives of Toxicology* 2020, 94 (19).
[28]Ao, L.; Fang, S.; Zhang, K.; Gao, Y.; Cui, J.; Jia, W.; Shan, Y.; Zhang, J.; Wang, G.; Liu, J., Sequence-dependent synergistic effect of aumolertinib-pemetrexed combined therapy on EGFR-mutant non-small-cell lung carcinoma with pre-clinical and clinical evidence. *Journal of experimental & clinical cancer research : CR* 2022, 41 (1), 163.

line 102-103: The author should indicate any criteria for selecting articles for full-text review.
Reply: Thanks for your valuable comments. We have modified our text as advised (see Page 6, line 102-106).
Changes in the text: We search for relevant literature written in English using the keywords "EGFR-TKIs", "combination therapy", "EGFR-mutated NSCLC", "acquired resistance", "clinical trial". The research selection process was divided into the following 3 stages: title review, abstract review, and full-text review. Original articles and review articles appropriate to the topic of this review were included in the full-text review phase.

line 112: The authors should cite, if any, appropriate references.
Reply: Thanks for your valuable comments. We have added references to this section for better information (see Page 6, line 116 and Ref 30).
Changes in the text:
[30] Appleman, L. J., MET signaling pathway: a rational target for cancer therapy. *Journal of clinical oncology* 2011, 29 (36), 4837-4838.

line 118-119: Is there any information available on the frequency of EGFR-mutant NSCLC with MET amplification?
Reply: Thanks for your valuable comments. In fact, MET amplification is the second most common mechanism of resistance to EGFR-TKIs.
References:
[1] Choi, Y. R.; Kang, E. H.; Kim, S.; Park, S. Y.; Han, J. Y.; Lee, Y., Single targeting of MET in EGFR-mutated and MET-amplified non-small cell lung cancer. *British Journal of Cancer* 2023, 128 (12), 2186-2196.
[2] Roper, N.; Brown, A. L.; Wei, J. S.; Pack, S.; Trindade, C.; Kim, C.; Restifo, O.; Gao, S.; Sindiri, S.; Mehrabadi, F., Clonal Evolution and Heterogeneity of Osimertinib Acquired Resistance Mechanisms in EGFR Mutant Lung Cancer. *Cell Reports Medicine* 2020, 1, 100007.

line 343 What was the incidence of excessive short-term gastrointestinal toxicity leading to early study closure?

Reply: Thanks for your valuable comments. Based on a pre-planned conventional definition of unacceptable toxicity frequency of 33%, this trial was closed to accrual for patient safety because the toxicity risk was 36%. We have modified our text as advised (see Page 18, line 366-367).
Changes in the text: This combination was associated with excessive short-term gastrointestinal toxicity (36%), which exceeded the pre-planned conventional definition of unacceptable toxicity frequency of 33% , leading to early study closure.

line 353 What was the incidence of interstitial pneumonia in this context?
Reply: Thanks for your valuable comments. Oxnard et al. found that there was a higher than expected frequency of interstitial lung disease (ILD) (22%). (see Page 17, line 358).

Reviewer B

This review titled "Clinical advances of EGFR-TKIs combination therapy in EGFR-mutated non-small cell lung cancer (NSCLC)" is talking about some combination strategies with EGFR-TKIs plus other treatment in NSCLC. This is a very general topic. Some major concerns:
1. Abstract – background: the description in the background is long but not specific to the title. The authors may want to introduce current statement of EGFR-TKIs in targetable NSCLC and the purpose and main goal of this review.
Reply: Thank you for your kind suggestion. We couldn't agree more with you. We have modified our text as advised (see Page 2, line 19-25).
Changes in the text: Mutations located in epidermal growth factor receptor (EGFR) tyrosine kinase domains have been demonstrated as the 'Achilles heel' of non-small lung cancer (NSCLC), and can be well targeted by epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). However, the clinical benefits of EGFR-TKIs are limited and acquired resistance to these drugs occurred inevitably in NSCLC patients after long-term exposure. In order to overcome this issue, EGFR-TKIs combination therapy appears to be a promising strategy, including combined targeted therapy, radiotherapy, chemotherapy and immunotherapy.

2. Abstract – findings: the contents listed here are too general. From the text presented by the authors, the readers might think this review is going to discuss the activities and toxicities. Obviously, the toxicity is not a main topic in this review. My understanding is the authors are trying to answer these questions: a. who should be treated with combination therapy? b. when should add the plus therapy to the EGFR-TKIs; c. what is the efficacy by using the combination therapy with potential more adverse effects?
Reply: Thank you for your kind suggestion. As Reviewer A pointed out that " It appears to be a repetition of the sentence in Key Content and Findings" , after careful consideration, we have modified our text as advised (see Page 2, line 32-35).
Changes in the text: In this review, we summarize EGFR-TKIs combination strategies,

including combined targeted therapy, radiotherapy, chemotherapy and immunotherapy, most of which have shown efficacy and safety in patients with EGFR-mutated NSCLC. More clinical studies with large sample sizes to analyze the activity and toxicity of combination therapy are necessary to explore potential and well-tolerated options.

3. In the main body of this review, the authors covered most of the combination strategies we commonly used in clinic. Some other combination therapies which are not included but also important: EGFR-TKIs + RET inhibitor (PMID: 36996322); EGFR-TKIs + other antibodies besides the PD-1/L1 or CTLA-4, such as anti-VEGF or anti-EGFR receptors; any other driver mutations, like KRAS or BRAF.

Reply: Thank you for your kind suggestion. Your comment is very significant for us. We have modified our text as advised (see Page 13, line 249-267 and Page 18, line 373-381).

Changes in the text:

3.6 Targeting RET

The RET proto-oncogene encodes a transmembrane receptor tyrosine kinase that is involved in normal embryonic development [73]. Due to an aberrant DNA repair process, the fusion of RET with another irrelevant gene occurs [74], which activates various downstream signaling cascades that play essential roles in cell proliferation and survival, namely PI3K/AKT, JAK2/STAT3 pathways [75]. It has emerged as a rare but targetable acquired resistance mechanism in EGFR-mutated NSCLC patients on EGFR-TKI treatment [76, 77].

Therefore, combination therapy with anti-EGFR and anti-RET therapy will be likely required to overcome this resistance. Notably, two highly potent RET inhibitors, selpercatinib and pralsetinib, have been proved for advanced or metastatic RET-altered NSCLC [77]. Meanwhile, RET fusions were more likely associated with EGFR-mutant NSCLC patients who received therapeutic interventions targeting EGFR with third-generation EGFR-TKIs [77]. In a multicenter, prospectively treated cohort, Rotow et al. indicated that the addition of selpercatinib to osimertinib was feasible and safe and offered clinical benefit for patients with EGFR-mutant NSCLC with an acquired RET fusion [78]. Besides, Piotrowska et al. reported that RET fusions mediate resistance to EGFR inhibitors and demonstrated that combined EGFR and RET inhibition with osimertinib/ pralsetinib (BLU-667) may be a well-tolerated and effective treatment strategy for EGFR-mutant NSCLC [79]. Urbanska et al. also found that EGFR-mutated patient has been displaying sustained ongoing OR to the osimertinib-pralsetinib combination for more than 12 months, providing clinical evidence for effectively targetable mechanism of osimertinib resistance [80].

References:

[73]Drilon, A.; Oxnard, G. R.; Tan, D. S.; Loong, H. H.; Johnson, M.; Gainor, J.; McCoach, C. E.; Gautschi, O.; Besse, B.; Cho, B. C., Efficacy of selpercatinib in RET fusion–positive non–small-cell lung cancer. *New England Journal of Medicine* 2020, 383 (9), 813-824.

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A.; Lipson, D.; Cronin, M. T.; Bloom, T.; Peretz, T., Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nature medicine* 2012, 18 (3), 382-384.
[75] Thein, K. Z.; Velcheti, V.; Mooers, B. H. M.; Wu, J.; Subbiah, V., Precision therapy for RET -altered cancers with RET inhibitors. *Trends in Cancer* 2021, 7 (12), 1074-1088.
[76] Zhu, V. W.; Klempner, S. J.; Ou, S. H. I., Receptor Tyrosine Kinase Fusions as an Actionable Resistance Mechanism to EGFR TKIs in EGFR -Mutant Non-Small-Cell Lung Cancer. *Trends in Cancer* 2019, 5 (11), 677-692.
[77] Wang, C.; Zhang, Z.; Sun, Y.; Wang, S.; Wu, M.; Ou, Q.; Xu, Y.; Chen, Z.; Shao, Y.; Liu, H., RET fusions as primary oncogenic drivers and secondary acquired resistance to EGFR tyrosine kinase inhibitors in patients with non-small-cell lung cancer. *Journal of Translational Medicine* 2022, 20 (1), 1-13.
[78] Rotow, J.; Patel, J. D.; Hanley, M. P.; Yu, H.; Awad, M.; Goldman, J. W.; Nechushtan, H.; Scheffler, M.; Kuo, C. S.; Rajappa, S.; Harada, G.; Clifford, S.; Santucci, A.; Silva, L.; Tupper, R.; Oxnard, G. R.; Kherani, J.; Drilon, A., Osimertinib and selpercatinib efficacy, safety, and resistance in a multicenter, prospectively treated cohort of EGFR-mutant and RET fusion-positive lung cancers. *Clinical Cancer Research* 2023, OF1-OF9.
[79] Piotrowska, Z.; Isozaki, H.; Lennerz, J. K.; Gainor, J. F.; Lennes, I. T.; Zhu, V. W.; Marcoux, N.; Banwait, M. K.; Digumarthy, S. R.; Su, W.; Yoda, S.; Riley, A. K.; Nangia, V.; Lin, J. J.; Nagy, R. J.; Lanman, R. B.; Dias-Santagata, D.; Mino-Kenudson, M.; Iafrate, A. J.; Heist, R. S.; Shaw, A. T.; Evans, E. K.; Clifford, C.; Ou, S. I.; Wolf, B.; Hata, A. N.; Sequist, L. V., Landscape of Acquired Resistance to Osimertinib in EGFR-Mutant NSCLC and Clinical Validation of Combined EGFR and RET Inhibition with Osimertinib and BLU-667 for Acquired RET Fusion. *Cancer Discov* 2018, 8 (12), 1529-1539.
[80] Urbanska, E. M.; Sørensen, J. B.; Melchior, L. C.; Costa, J. C.; Santoni-Rugiu, E., Durable Response to Combined Osimertinib and Pralsetinib Treatment for Osimertinib Resistance Due to Novel Intergenic ANK3-RET Fusion in EGFR-Mutated Non-Small-Cell Lung Cancer. *JCO precision oncology* 2022, 6, e2200040.

In addition, high levels of circulating vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis, which plays an important role in growth, proliferation and metastasis of tumor cells in NSCLC patients [116]. ARTEMIS-CTONG1509, a multicenter phase 3 study, indicated that bevacizumab (an anti-VEGF antibody) plus erlotinib significantly improved PFS in patients with EGFR-mutated NSCLC, including those with brain metastases at baseline [117]. Kuo et al. found that bevacizumab combination treatment showed moderate efficacy in afatinib-treated NSCLC patients with EGFR-sensitizing mutation [118]. However, treatment with osimertinib plus bevacizumab failed to exhibit the efficacy for improving the PFS among EGFR-mutated NSCLC patients, which have been reflected in the studies of Soo et al. and Kenmotsu et al [119,120].

References:

[116] Peravali, M.; Wang, H.; Kim, C.; Veytsman, I., Combined Inhibition of EGFR and VEGF Pathways in Patients with EGFR-Mutated Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis. *Current oncology reports* 2020, 22 (12), 119.
[117] Zhou, Q.; Xu, C. R.; Cheng, Y.; Liu, Y. P.; Chen, G. Y.; Cui, J. W.; Yang, N.; Song, Y.; Li, X. L.; Lu, S.; Zhou, J. Y.; Ma, Z. Y.; Yu, S. Y.; Huang, C.; Shu, Y. Q.; Wang, Z.; Yang, J. J.; Tu, H. Y.; Zhong, W. Z.; Wu, Y. L., Bevacizumab plus erlotinib in Chinese patients with untreated, EGFR-mutated, advanced NSCLC (ARTEMIS-CTONG1509): A multicenter phase 3 study. *Cancer Cell* 2021, 39 (9), 1279-1291.
[118] Kuo, C. S.; Chiu, T. H.; Tung, P. H.; Huang, C. H.; Ju, J. S.; Huang, A. C.; Wang, C. C.; Ko, H. W.; Hsu, P. C.; Fang, Y. F.; Guo, Y. K.; Yang, C. T., Afatinib Treatment Alone or with Bevacizumab in a Real-World Cohort of Non-Small Cell Lung Cancer Patients with Epidermal Growth Factor Receptor Mutation. *Cancers (Basel)* 2022, 14 (2), 316.
[119] Soo, R. A.; Han, J. Y.; Dafni, U.; Cho, B. C.; Yeo, C. M.; Nadal, E.; Carcereny, E.; de Castro, J.; Sala, M. A.; Bernabé, R.; Coate, L.; Provencio Pulla, M.; Garcia Campelo, R.; Cuffe, S.; Hashemi, S. M. S.; Früh, M.; Massuti, B.; Garcia-Sanchez, J.; Dómine, M.; Majem, M.; Sanchez-Torres, J. M.; Britschgi, C.; Pless, M.; Dimopoulou, G.; Roschitzki-Voser, H.; Ruepp, B.; Rosell, R.; Stahel, R. A.; Peters, S., A randomised phase II study of osimertinib and bevacizumab versus osimertinib alone as second-line targeted treatment in advanced NSCLC with confirmed EGFR and acquired T790M mutations: the European Thoracic Oncology Platform (ETOP 10-16) BOOSTER trial. *Annals of Oncology* 2022, 33 (2), 181-192.
[120] Kenmotsu, H.; Wakuda, K.; Mori, K.; Kato, T.; Sugawara, S.; Kirita, K.; Yoneshima, Y.; Azuma, K.; Nishino, K.; Teraoka, S.; Shukuya, T.; Masuda, K.; Hayashi, H.; Toyozawa, R.; Miura, S.; Fujimoto, D.; Nakagawa, K.; Yamamoto, N.; Takahashi, T., Randomized Phase 2 Study of Osimertinib Plus Bevacizumab Versus Osimertinib for Untreated Patients With Nonsquamous NSCLC Harboring EGFR Mutations: WJOG9717L Study. *Journal of Thoracic Oncology* 2022, 17 (9), 1098-1108.

4. The decision making of treatment with EGFR-TKIs plus other therapy is mainly because of resistance mechanism. I suggest the authors add a section discussing this important point, including both de novo pathways and acquired resistant mechanism for EGFR-mutant NSCLC treated with EGFR-TKIs. And how do these mechanisms drive the patient selection in clinical practice?

Reply: Thank you for your kind suggestion. Our manuscript focuses on EGFR-TKIs combination therapy, including combined targeted therapy, radiotherapy, chemotherapy and immunotherapy. As for various EGFR-TKI resistance mechanisms, this is the planned topic of our next review, which we desire to introduce them in detail and systematically as much as possible.