Peer Review File

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Reviewer A

Comment 1: The one concern is that there may be overlaps for resistant mutations that are reported for osimertinib that can result in resistance to afatinib. These can include MET amplification, T790M, C797X, MET/HER2, RAS, MAPK, and PI3K mutations. More genotyping via NGS can help focus the patient cohort to work on The authors have to address this point critically.

Reply 1: We fully agree with your comment. Ideally, we also believe that the corresponding targeted therapy should be provided for each resistance mechanism that emerged following osimertinib. As you mentioned, afatinib might potentially be resistant to some off-target resistance mechanisms. However, given that resistance mechanisms are unknown in about 50% of patients after first-line osimertinib (1), we thought that afatinib rechallenge should be evaluated in all patients after osimertinib resistance, not just those with some resistance mechanisms. Besides, due to the considerable amount of turn-around time with CGP testing, we recognize that a next-line regimen is generally decided irrespective of CGP test results in practice, except in the case of participation in particular clinical trials. Thus, in our study, though undergoing CGP testing before enrollment is set as one of the inclusion criteria, it is not necessary to confirm the results. Of course, if other treatment recommendations were provided by the expert panel discussion based on their CGP test results, patients were assured to arbitrarily choose those treatments or participate in specific clinical trials. The lack of subgroup analysis to verify the efficacy of afatinib based on molecular profiling due to a small sample size is a serious concern of the study. Thus, we added some additional descriptions in the “Discussion” (please see the Page 11, line 219~222.).

Change in the text: Page 11, line 219~222. (highlighted in red)

Comment 2: is the focus just for a study in Japan as noted in line 221?

Reply 2: Yes, it is. This study is being conducted exclusively in Japan, and 11 facilities will be participating.

Change in the text: None.

Reviewer B

Comment 1: The data that the authors cite as their rationale for conducting this study support the hypothesis that afatinib will be effective specifically among patients with EGFR on-target resistance mechanisms. However, their study plans to accrue all patients who developed resistance to first-line osimertinib regardless of resistance mechanism. Can the authors explain why there are no pre-specified secondary endpoints that will evaluate the efficacy of afatinib in this particular patient subset?

Reply 1: As you indicated, we are also very interested in the efficacy of afatinib in patients with acquired on-target resistance mechanisms. However, given the scale of our study, it was difficult to plan a pre-specified subgroup analysis because we could not estimate how many patients with on-target resistance mechanisms would be recruited. Instead, we are going to individually review the molecular profile information and efficacy of afatinib apart from the analysis of study endpoints. The lack of subgroup analysis to verify the efficacy of afatinib based on molecular profiling due to a small sample size is a serious concern of the study. Thus, we added some additional descriptions in the "Discussion" (please see the Page 11, line 219~222.).

Change in the text: Page 11, line 219~222. (highlighted in red)

Comment 2: The authors discuss the efficacy of dacomitinib, another second-generation pan-ErbB family TKI, which was evaluated in a phase II prospective study after resistance to first-line osimertinib, and achieved a modest ORR of 16.7%. This falls below the alternative hypothesis of 30% that the authors are aiming to achieve in this study. Do the authors have rationale for why afatinib would be more effective than dacomitinib in achieving this ORR target?

Reply 2: Thank you very much for your precise comment. To our knowledge, there are no clinical data comparing the superiority of afatinib to dacomitinib, including retrospective studies, in any treatment setting. As you mentioned, the ORR for dacomitinib reported by Choudhury *et al.* (2) cited in the manuscript was modest, but only 12 patients were recruited when 24 were to be enrolled. Furthermore, in their study, 82% of patients had *TP53* co-mutation, a known poor predictive factor of EGFR TKI efficacy, before dacomitinib induction. This is a relatively higher frequency of *TP53* mutation after osimertinib resistance than the previously reported value (1) and may have led to the disappointing ORR of dacomitinib in the study population. Thus, we believe that the efficacy of second-generation EGFR TKI rechallenge after 1L osimertinib needs to be investigated in another cohort. The alternative hypothesis for the ORR of afatinib monotherapy in our study was determined concerning data from retrospective studies on second-generation EGFR TKI rechallenge (3-5) and the efficacy data of docetaxel plus ramucirumab (DTX + RAM) regimen, which is assumed to be a standard later-line regimen for advanced *EGFR*-mutated patients followed by second-line platinum-based chemotherapy. This is because our study was designed to enroll patients in the third-line setting. Thus, we thought the expected response rate for afatinib should be set based on these regimens. The reported ORR of afatinib rechallenge after first- and second-generation EGFR TKIs was about 25% (3,4). In the population that included patients previously treated with osimertinib, the ORR for dacomitinib rechallenge was reported to be 25% (5). Moreover, the ORR for DTX + RAM in Japanese patients was reported to be 28.9% (6). Based on these preceding data, we set the expected response rate for afatinib at 30%. We added some additional descriptions in the "Discussion" (please see the Page 10, line 196~203.).

Change in the text: Page 10, line 196~203. (highlighted in red)

Comment 3: Afatinib is effective in treating EGFR-mutated NSCLC following acquired resistance to first-generation EGFR TKIs only when delivered in combination with the EGFR antibody cetuximab. Can the authors explain their rationale for evaluating only single-agent afatinib after acquired resistance to osimertinib?

Reply 3: At present, there is no rational data that afatinib monotherapy is effective after 1L osimertinib resistance. We also understand that the afatinib and cetuximab combination regimen might be more effective against osimertinib resistance than afatinib monotherapy. While the combination strategy is an attractive treatment candidate, it is not an approved use in Japan. The aim of our study is to provide evidence for the EGFR TKI rechallenge, which is being challenged individually in clinical practice. Considering that a modest proportion of patients were given EGFR TKI followed by 1L osimertinib in the FLAURA trial, we thought that exploring the efficacy of a rechallenge regimen in this population would be of clinical importance.

Changes in the text: None.

Reviewer C

Comment 1: Even though EGFR-TKI showed promising survival benefit, resistance to EGFR-TKI eventually developed in most of the patients. After failing EGFR-TKI, cytotoxic chemotherapy or other agents such as antibody-drug conjugate, other biologic agents were used as subsequential treatment. This study plans to evaluate the efficacy of afatinib rechallenge. Except for chemotherapy, there are limited options for subsequential treatment after failing osimertinib. I agreed with the unmet medical need for this study population. The abstract is well-written for the purpose of this study.

Reply 1: Thank you very much for your cooperation in reviewing the manuscript of our study protocol.