Peer Review File

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Reviewer A

Major comments

1. (introduction) To date, other therapeutic strategies option has been developed for first-line treatment of EGFR-mutated NSCLC patients, including initial combined treatment with anti-angiogenesis agent plus chemotherapy to overcome above issues and other EGFR-TKIs (6,7).
= optionS and agentS (or AN agent)
> I would debate this in the discussion; in addition, the authors refer two strategies which are being explored to overcome resistance/improve PFS: (1) combination with an anti-angiogenesis agent (reference 6 = erlotinib + ramucirumab), although this is also being explored with Osimertinib; (2) combination with chemotherapy (reference 7 = gefitinib + chemotherapy), again not with Osimertinib; no reference/details are given about new TKIs.

Reply: We agree with the reviewer's suggestion that multiple promising therapeutic strategies are being developed for untreated advanced NSCLC patients with EGFR mutations. Therefore, we have modified our text as advised (see Page 6, line 17).

Changes in the text:

To date, other therapeutic strategies have been approved in several countries, including the USA and Japan, for first-line treatment of EGFR-mutated NSCLC patients, including initial combination therapy with an anti-angiogenesis agent and chemotherapy to overcome the above issues and other EGFR-TKIs (6,7).

2. (introduction) Recently, immune-checkpoint inhibitor (ICI) therapy has made rapid advances in several cancers, including lung cancer, according to improved clinical outcomes, such as prolonged survival and a more durable treatment response (8-12).
> here 5 references are used to discuss something in a really vague way. I personally would rewrite the introduction to give an overview of the current treatment field of EGFRm+ NSCLC, with current challenges: primary resistance, secondary resistance, … and what is known about PD-L1 in EGFRm+.

Reply: As reviewer A suggested, we have added a sentence about primary resistance, acquired

resistance of EGFR-TKI treatment (Page 7, lines 4-14), and activation of EGFR signaling pathways involved in the production of PD-L1 expression(Page 8, lines 6-8).

Changes in the text:

(Page 7, lines 4-14) Although *EGFR*-mutated advanced NSCLC cells respond well to osimertinib initially, a small percentage of cells can survive and expand, leading to acquired drug resistance and tumor heterogeneity, ultimately promoting tumor recurrence. As for intrinsic resistance to EGFR-TKIs, *EGFR*-T790M mutation, EGFR-exon20 insertions, and BIM deletion polymorphism have been reported as contributory factors (8-10). Based on previous reports, acquired-resistance mechanisms can be broadly classified into resistance caused by the treatment target EGFR [*EGFR*-T790M secondary resistance gene mutation (11)], resistance via non-EGFR bypass signal [Met gene amplification (12), HGF overexpression (13), HER2 gene amplification (14), GAS6-AXL signal activation (15)], and other resistance [transformation to small cell lung cancer (16) and epithelial-to-mesenchymal transition (17)].

(Page 8, lines 6-8)) Preclinical studies have shown that activation of EGFR signaling pathways is involved in the induction of PD□L1 expression in NSCLC cells (25).

3. (introduction) Additionally, tumor PD-L1 expression is reportedly a negative prognostic factor for response to docetaxel treatment by NSCLC patients according to a clinical trial.
> I cannot recall having seen this in the results of this trial. There was a slightly worse OS and PFS in high PD-L1, but I don't think this was significant. Could the authors clarify this statement?

Reply: We agree with the reviewer's opinion and deleted the sentence regarding the efficacy of docetaxel on PD-L1 expression levels.

Changes in the text:

We deleted the following sentences: Additionally, tumor PD-L1 expression is reportedly a negative prognostic factor for response to docetaxel treatment by NSCLC patients according to a clinical trial.

4. (methods) This study prospectively records the response to 1L osimertinib in EGFRm+ NSCLC (n=71) in 17 hospitals Japan.
> Were these consecutive patients or a random sample?

Reply: In this study, we assessed all consecutive patients who fit the inclusion criteria, and only

patients who provided written informed consent were enrolled, as shown in supplementary Figure 1.

> More information on inclusion criteria should be given, even when a table of patient characteristics is available: e.g. where untreated, symptomatic brain metastases allowed, which performance status was allowed (I notice WHO 3 is allowed), …?

Reply: As the reviewer recommended, we added the information on inclusion criteria in the methods section (see Page 9, lines 13-18).

Changes in the text:
The inclusion criteria in this study are as follows; 1) patients without any systemic treatment, 2) symptomatic brain metastases are allowed, 3) any Eastern Cooperative Oncology Groups performance status (ECOG PS) is allowed, 4) EGFR mutations, including L858R point mutation in exon 21 and exon 19 deletions, in addition to the other types of mutation, such as G719X in exon18, S768I in exon 20, L861Q in exon 21, were included.

> ORR should not be reported in this table of patient characteristics; this belongs under results.

Reply: We removed the results of the objective-response rate and disease-control rate for osimertinib treatment in Table1 and moved it to supplementary Table1 (see Page 13, lines 14-17).

Changes in the text:
We then examined the effect of tumor PD-L1 expression on osimertinib efficacy. In all *EGFR*-mutated NSCLC patients, the objective-response rate (ORR) and disease-control rate (DCR) for osimertinib treatment were 72.1% and 92.6%, respectively **(Supplementary Table 1).**

> Why did the authors include not only the classical L858R point mutation (exon 21) and exon 19 deletions, but also the G719C (exon 18) mutation? This mutation was not allowed in the FLAURA trial and Osimertinib is not registered for this mutation in all countries. The text should include which mutations were allowed (e.g. other mutations in exon 21 allowed but not discovered, or not allowed?).

Reply: As the reviewer recommended, we added the information regarding EGFR mutation status and inclusion criteria in the methods section (see Page 9, lines 13-18).

Changes in the text:

The inclusion criteria in this study are as follows; 1) patients without any systemic treatment, 2) symptomatic brain metastases are allowed, 3) any Eastern Cooperative Oncology Groups performance status (ECOG PS) is allowed, 4) EGFR mutations, including L858R point mutation in exon 21 and exon 19 deletions, in addition to the other types of mutation, such as G719X in exon18, S768I in exon 20, L861Q in exon 21, were included.

> Why did the authors use PCR to detect EGFR mutations, given the availability of NGS in 2019-2020? Presence of mutations in other genes might be relevant for (primary) resistance to Osimertinib. The poorer performance of patients with high PD-L1 expression might be due to co-mutation, rather than PD-L1 status per se.

Reply: We agree with the reviewer's comment that PCR methods are relatively underpowered, making it an inferior method to detect compound mutations of EGFR genes compared with NGS analysis. Therefore, we added the sentence regarding this point as a limitation of our study in the discussion (see Page 19, lines 11-13).

Changes in the text:

Third, *EGFR* mutation status was detected using PCR analysis, which has limitations in the detection of compound mutations.

5. (results) We assessed correlations of clinicopathological features by comparing the PD-L1 groups, which revealed that patients with EGFR uncommon mutation were significantly more common in the PD-L1-high group than in the PD-L1-low and -negative groups
> This was based on results from 3 patients! The likelihood that this is a coincidence is high.

Reply: We agree with the reviewer's suggestion and deleted the sentence in the results section as the patient number was too small to evaluate statistical significance (see Page 13, lines 11-13).

Changes in the text:

We assessed correlations of clinicopathological features by comparing the PD-L1 groups. There was no significant difference between the three groups **(Table 4)**.

6. Among the 70 EGFR-mutant NSCLC patients showing disease progression within 90 days or during >90-day follow-up, seven were identified as exhibiting primary resistance to osimertinib treatment and categorized as disease progression within 90 days.

> Primary resistance should be defined in the methods, not in the results section.

Reply: We have modified this information in the methods section (see Page 9, lines 10-13).

> Disease progression within 90 days or during >90-day follow-up = just disease progression

Reply: We deleted these words as advised (see Page 9, lines 10-13).

Changes in the text:
Among the 70 *EGFR*-mutant NSCLC patients showing disease progression within 90 days or during > 90-day follow-up, seven were identified as exhibiting primary resistance to osimertinib treatment and categorized as "disease progression within 90 days".

Minor comments
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1. (introduction) majority subtypes
= major subtypes
Reply: we have modified the text as advised (see Page 6, line 5).

Changes in the text:
Improved clinical outcomes in NSCLC patients harboring *epidermal growth factor receptor* (*EGFR*) mutations, including major subtypes, such as (…).

2. (introduction) improved clinical outcomes are attributed to (…)
> I think there is ample evidence that the improved clinical outcome results from the administration of EGFR-TKIs; the word attribution creates a relation of uncertainty which is no longer present
Reply: We corrected "attributed" to "contributed" as advised (see Page 6, lines 6-7).

Changes in the text:
improved clinical outcomes have contributed to (…).

3. (introduction) better respond
= better responses
Reply: we have modified our text as advised (see Page 6, line 8).

Changes in the text:
NSCLC patients with activating *EGFR* mutations showed better responses to (…).

4. (introduction) wildy type NSCLC
= wild-type NSCLC
Reply: we have modified our text as advised (see Page 8, line 3).

Changes in the text:
especially for NSCLC patients with wild-type driver oncogenes (…).

5. (results) in 71 EGFR-mutant advanced NSCLC patients with PD-L1 IHC test this suggests more a retrospective approach in which only EGFRm+ with a known PD-L1 were included; if it was prospective, all patients must have had a PD-L1 IHC, no?

Reply: In this study, we planned to prospectively evaluate the role of tumor PD-L1 expression on the efficacy of osimertinib among EGFR mutated NSCLC patients. We added the CONSORT diagram as the supplementary Figure 1 to help readers understand (see Page 12, lines 14-15).

Changes in the text:
Fifty-four patients were followed up for more than 1 year, and 3 patients for more than 2 years **(Supplementary Fig. 1)**.

6. (results) It looks a bit peculiar that only 56% of patients with EGFRm+ were non-smokers?

Reply: As reviewer pointed out, in general, NSCLC patients with EGFR mutation are more likely to be non-smokers. However, smoking status was not an independent prognostic factor for efficacy of osimeritnib, even though there were many smokers in this study, indicating that smoking status was not as important for the outcomes of osimertinib as tumor PD-L1 expression.

Reviewer B

The authors investigated the effect of tumor PD-L1 level on the efficacy of osimertinib monotherapy in EGFR mutant lung cancer in Japanese hospitals. The findings are in line with previous studies that showed a poor prognosis of PD-L1 expressed lung cancer treated with

EGFR-TKIs. However, there are some points that authors should clarify for further publications.

Major points

1. The small number of study subjects and relatively short follow-up time are significant limitations of the study. Although authors have written as prospective study design, the patients' enrollment was done between September 2019 and December 2020. Therefore, some patients have a follow-up time of fewer than six months.

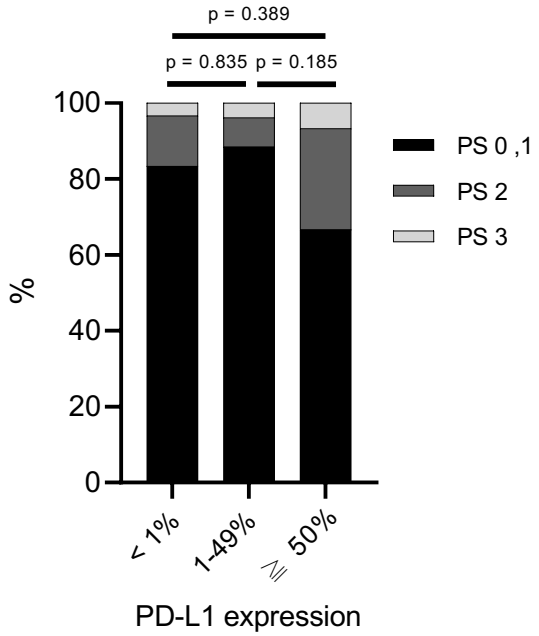
Reply: In this study, the 2 patients with a follow-up time of less than six months were enrolled. Therefore, we added this information as a limitation of our study in the discussion part (see Page 19, lines 12-13).

Changes in the text:
Finally, two patients with a follow-up time of less than six months were enrolled.

2. In the FLAURA study, a prospective phase III trial, PD-L1 expression was not predictive of response in patients (200 patients) with osimertinib treatment (Brown et al. J Thorac Oncol 2020;15:138-43). Therefore, I think there should be a more in-depth analysis by the authors or unique information to publish the current findings, such as the difference between the current study vs. FLAURA study, preclinical mechanism study of resistance in PD-L1 high tumor, and translational research findings). Or else, convincing the readers of the current results will be very difficult due to study limitations as wrote above. (I am wondering if some patients with poor PS and short survival time are incidentally included in the PD-L1 high group) I think ECOG PS is a prognostic factor and not a predictor of EGFR-TKI treatment response according to the previous studies.

Reply: In the FLAURA study, tumor PD-L1 expressions were divided into two groups, ≧1% and <1%, resulting in the failure of tumor PD-L1 expression to predict osimertinib response. In this study, data showed that only tumors with PD-L1 expression ≧ 50% were a poor predictive factor for osimertinib treatment, compared to tumors with 1-49% and <1% of PD-L1 expression. We think that these findings do not contradict those of the FLAURA study because PD-L1 expression was evaluated based on different criteria in our study. We explained this in the discussion section (see Page 16, lines 16-17). In addition, we analyzed the relationship of ECOG PS and tumor PD-L1 expression as the reviewer recommended, and the correlation of poor PS and high PD-L1

expression was not significant in this study, as given below (for the reviewer only).



For the reviewer only: the correlation of PS status and PD-L1 expression in this study

Changes in the text:

These results suggest that tumor PD-L1 expression of ≥ 50% might be a potent negative prognostic factor for EGFR-TKI treatment.

3. The study was registered and released on 15th April 2021 at the trial registry site (https://upload.umin.ac.jp/cgi-open-bin/icdr_e/ctr_view.cgi?recptno=R000050167). So, It would be good to show other evidence that the trial initiation and patients enrollment was started prospectively before September 2019.

Reply: We had performed opt-out informed consent in each hospital from beginning of the trial. We mentioned this information in Ethical Statement section (see Page 22, lines 4-5) and the methods section (see Page 10, lines 4-6).

Changes in the text:

In addition, we had performed opt-out informed consent in each hospital from the beginning of

the trial.

Minor points

1. Overall survival time and successive treatment results should be included in the study.

Reply: We added overall survival time and successive treatment results in the results section (see Page 12, lines 15-16) (see Page 15, lines 4-6) and Supplementary Figure 2.

Changes in the text:

(Page 12, lines 15-16) Median overall survival time (OS) was not evaluable (NE) (95% CI: 22.4 months–NE) **(Supplementary Fig. 2A)**, and successive treatment was in 17 patients (23.9%).

(Page 15, lines 4-6) There was no significant relationship in OS between PD-L1-high patients and PD-L1-low plus -negative patients (p = 0.858) **(Supplementary Fig. 2B)**.

2. The CONSORT diagram is not included in the manuscript. The authors should include information about screening failure or reasons for off-study (progression and death).

Reply 2: As reviewer recommended, we added the CONSORT diagram in the results section (see Page 12, lines 14-15) and Supplementary Fig. 1.

Changes in the text: The median follow-up time for this study was 15.5 months (range: 1.2–25.1 months). Fifty-four patients were followed up for more than 1 year, and 3 patients for more than 2 years **(Supplementary Fig. 1)**.