Peer Review File
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Reviewer A

Gilardone et all reported retrospective real-world data on osimertinib versus afatinib as first-line treatment for patients with classical EGFR mutated NSCLC. Their findings were no difference in PFS in the real-world setting. It is important to analyze these data in real-world patient to provide more information and treatment options for clinicians all over the world. The data is clear described. There are some minor revisions:

We appreciate the reviewer highlighting the need for real-world information regarding this topic and for the positive comment that data was clearly described. We also thank them for their assistance and suggestions to improve the manuscript which we have addressed below.

Comment 1: In the discussion the authors state that patients being treated in study setting have a lower eligible change of treatment with a 2nd line TKI. In your real-world data, much more patients were eligible for 2nd line TKI. Do you have an idea of the reason for this? And can you state that as well in the discussion.

Reply 1: We thank the reviewer for highlighting the difference between previous study findings and our findings. We have included a possible explanation in our discussion.

Changes in the text: "Previous real-world studies suggest that ~30% of the patients will be eligible for second-line therapy with a EGFR TKI, which has been theorized to be due to the rapid progressive nature of the disease leaving patients unable to proceed with subsequent therapy. [16–18] This is much lower than what we report in our study. Possible explanations could be higher rates of genomic testing at the time of disease progression at a high-volume academic center such as ours or possibly due to the small number of patients included in the afatinib group in this study."(see pages 11-12, lines 264-270)

Comment 2: Make sure to add a capture for every table/figure, including abbreviations.

Reply 2: We thank the reviewer for this request. All tables and figures have captions that have been added. Where necessary, abbreviations were defined.

Changes in the text: Tables 1 -4, Figures 1 -3 (see page 17, lines 440-447)

Reviewer B

Gilardone S. et al. have raised an interesting and not fully clarified issue of comparing Osimertinib and Afatinib in the first line setting in patients with metastatic NSCLC with

sensitizing EGFR mutations: ex19 del and L858R. As the authors have recalled that there are no studies directly comparing the efficacy of these two drugs in the first-line setting, and they have presented a single-institution study to discuss this topic. Afatinib and Osimertinib have been retrospectively evaluated in terms of PFS, OS, median time on first line EGFR-TKI, and toxicity.
The article has a good introduction, where the authors pointed out the unresolved question whether Afatinib may also be a reasonable option in the first-line setting for patients with metastatic NSCLC with EGFR exon 19 deletion or L858R mutations.

There were 86 patients included in this study, and the number of patients in both comparing groups were unbalanced despite employing a match study design. Though, the distribution of other patients' characteristics were similar in both groups, apart from difference in patients with EGFR L858R mutation with only 7 % in Afatinib group versus 27% in Osimertinib group. Interestingly, both groups showed, at there were similarly about 30% patients eligible to the study despite the difference in the number of pre-screened patients, respectively 41 patents prescribed Afatinib, and 219 patients prescribed osimertinib.
The study showed no differences in the PFS and OS. Furthermore, as showed in the figure 2, most of the patients treated with Afatinib progressed faster in the first 12 months, while the patients treated with Osimertinib had a 12-month plateau. Both curves overlapped later on. However, a considerable difference between two groups in a median follow-up may have influence of assessing both PFS and OS.

Statistical analysis, patients' characteristics and outcome were well described and presented the tables.

Another important issue is activity of Osimertinib in the brain. It is its one of undisputable value of this EGFR-TKI. However, if Afatinib should be considered as an equivalent choice for Osimertinib in EGFR-mutated patients with brain metastases, some more discussion is needed to present this challenge. As presented in the table 1, presence or absence of baseline CNS metastases was not significant for treatment type (p value 0.757).
In patients with EGFR mutated NSCLC the incidence of brain metastasis is higher than the NSCLC patients with wild-type EGFR and EGFR-mutated NSCLC has a higher propensity to metastasize into the brain (1). Given the significant CNS efficacy of Osimertinib (2), and the fact that Osimertinib was the only EGFR-TKI of 12 tested to achieve significant brain penetrance, compared with the other twelve EGFR-TKIs (1), the question is whether it may be acceptable to use Afatinib and not covering the risk of CNS metastasizing. However, as showed in the table 3 – surprisingly - only Osimertinib patients developed CNS metastasis, especially in the aspect of longer follow-up in Afatinib group. Therefore, it is still challenging to identify patients most prone to develop spread in the CNS. Furthermore, there are some data showing better efficacy of Osimertinib versus 2. gen. EGFR-TKI for patients with brain metastases (3). On the other hand, there is another recently published retrospective analysis showing

efficacy of Afatinib in first line for EGFR-mutated NSCLC patients with brain metastases with the CNS ORR was 48.8% in the whole group of 43 patients and 82.6% in patients with measurable brain metastases (4). Moreover, Lu et al. have showed efficacy of Afatinib in elderly patients and in patients with PS $\geq$ 2 – both groups frequently excluded form clinical trials (5). Therefore, some more discussion is needed to shed light on this challenge, taking also in consideration that a sequential treatment with Afatinib followed by Osimertinib may result in longer time to treatment failure, especially in Asians patients

Despite quite similar toxicity, in this study 64% of Afatinib patients required dose reduction comparing with only 19% of Osimertinib patients. This difference is clinically important as it may influence quality of life of patients treated with Afatinib, which can be more highlighted in the study.

We are grateful to the reviewer for accurately summarizing our manuscript. We appreciate the positive feedback on the introduction and the comment that our manuscript provided a "good contribution to the discussion about the first-line EGFR-TKI in patients with Metastatic NSCLC with EGFR mutations". We are thankful to the reviewer for the suggestion to highlight more clearly the potential difference in quality of life between the groups in reference to the incidence of dose reductions. We thank the reviewer for the detailed analysis and helping to improve the manuscript with their comments which have been addressed below.

Other questions

Comment 1: What was the reason difference in median follow-up for these two groups: 56 versus 22 months?

Reply 1: We thank the reviewer for raising an important point regarding the difference between the groups. The FDA approved afatinib for clinical use in 2013 while osimertinib was approved in 2018 and the included patients were treated between 01/01/2023 and 04/30/2021. Also, there were 4/15 patients in the afatinib group that were still on afatinib at time of data cut off, which also contributed for the longer follow-up for these patients.

Changes in the text: "The extended follow up time in the afatinib group can potentially be explained by the earlier date of approval and treatment start date in the afatinib group starting years prior to osimertinib and that four of the fifteen patients treated with afatinib were still on therapy at time of data cut off." (see page 10, lines 230-233)

Comment 2: In seven patients progressing on Afatinib, an EGFR T790M mutation was confirmed. How was this finding confirmed: by tumour rebiopsy of cfDNA? Which NGS panel was applied? Were there other molecular alterations found beyond EGFR T790M?

Reply 2: We thank the reviewer for inquiring about identification of T790M mutations. The EGFR T790M mutation was confirmed via EGFR pyrosequencing in tumor tissue or liquid biopsy in blood plasma with Guardant 360® or droplet digital PCR (ddPCR) with Biodesix. We have added the table below for the reviewer only.

Study ID #	**Liquid Biopsy**	**Type of liquid biopsy assay**	**Assay**	**Tissue testing**	**Type of tissue testing**
3	Yes	ddPCR	Biodesix		
4	No			Yes	EGFR pyrosequencing*¥
7	No			Yes	EGFR pyrosequencing*
10	Yes	Target Hybrid-capture NGS	Guardant360		
12	No			Yes	EGFR pyrosequencing*
13	Yes	Target Hybrid-capture NGS	Guardant360¥	No	
15	No			Yes	EGFR pyrosequencing*
17	Yes	Target Hybrid-capture NGS	Guardant360		
19	Yes	Target Hybrid-capture NGS	Guardant360		

*This EGFR mutation test uses pyrosequencing technology, targeting mutations on four EGFR exons: 18, 19, 20, and 21.

¥ These tests were negative for an EGFR T790M

Changes in the text: "An EGFR T790M mutation was confirmed via *EGFR* pyrosequencing in tumor tissue or liquid biopsy in blood plasma with Guardant 360 (Target hybrid capture-based next generation sequencing) or Biodesix (droplet digital PCR)" (see page 9, lines 199-201)

Comment 3: The second line treatment after progression on Afatinib or Osimertinib

should be more clarified. How many patients were re-biopted and how they were treated in second line?

Reply 3: We appreciate the reviewer highlighting a need for clarification with second line therapy. However, since this project was focused on 1st-line treatment and time on EGFR TKI, we did not collect data on subsequent lines of therapy for patients treated with osimertinib in the first-line setting. We do have data on subsequent lines of therapy for patients treated with afatinib, which we summarized for the reviewer only in the table below. Regarding re-biopsy on the Afatinib group, only 8 patients were known to progress on Afatinib, and 50% of those underwent a re-biopsy. We have added information in the text to summarize the data presented in this table and also added the lack of information on subsequent lines of therapy for the entire study cohort as a limitation of the present study.

Study ID	**First-line treatment**	**Second-line treatment**	**Comments**
3	Afatinib	Osimertinib	
4	Afatinib	Osimertinib	
5	Afatinib	NA	Still on afatinib at the time of data cut off
7	Afatinib	Osimertinib+ mTOR Inhibitor	
10	Afatinib	Unknown	Lost to f/u
12	Afatinib	Osimertinib	
13	Afatinib	Osimertinib	
14	Afatinib	NA	Still on afatinib at the time of data cut off
15	Afatinib	Osimertinib	
16	Afatinib	Unknown	Lost to f/u
17	Afatinib	Osimertinib	
18	Afatinib	NA	Still on afatinib at the time of data cut off
19	Afatinib	Osimertinib	
20	Afatinib	Osimertinib	No PD on afatinib; switched due to AEs
21	Afatinib	NA	Still on afatinib at the time of data cut off

Changes in the text: "Osimertinib was selected as a second-line therapy in six of these seven patients. Additionally, three other patients were treated with osimertinib in the

second-line setting after Afatinib either due to AEs secondary to Afatinib or due to physician's choice upon progression even in the absence of a T790M mutation." (see page 9, lines 202-205)

"Data on second-line and subsequent therapy was not collected, so we are unable to conclude which EGFR sequencing strategy may be superior as this was not analyzed." (see page 13, lines 316-318)

Comment 4: Do you have any reflexion why patients in the Afatinib group were on 1st -line EGFR TKI therapy nearly 9 months longer than the ones in the Osimertinib group? May it reflect the observation that Afatinib is prone to be more effective for EGFR exon 19 deletion than for EGFR L858R substitution?

Reply 4: We appreciate the reviewer's suggestion to expand further as to why the time on TKI therapy may have been prolonged in the afatinib group. In addition to the points that were mentioned in the discussion, a statement was added proposing that because afatinib has been found to be more efficacious with exon 19 deletions compared to exon 21 L858R mutations, it could have affected the time on TKI therapy.

Changes in the text: "However, the latter was not statistically significant and could be related to the longer follow-up time of patients treated with afatinib, the small number of patients in the afatinib group, or possibly because the vast majority of patients treated with afatinib had an exon 19 deletion in which afatinib has found to be more effective in compared to the exon 21 L858R mutation.[9] The extended follow up time in the afatinib group can potentially be explained by the earlier date of approval and treatment start date in the afatinib group starting years prior to osimertinib and that four of the fifteen patients treated with afatinib were still on therapy at time of data cut off." (see page 10, lines 230-236)

To resume, I have found the manuscript interesting with a good contribution to the discussion about the first-line EGFR-TKI in patients with Metastatic NSCLC with EGFR mutations.

Reviewer C

Gilardone et al. conducted a retrospective analysis on 86 patients with the most common form of EGFR mutation-positive NSCLC, treated with afatinib or osimertinib. The added value of this study is very limited, given the retrospective nature and the limited number of patients (especially in the afatinib cohort). Furthermore, the median follow-up time is too short for osimertinib. Additionally, the toxicity handling was done poorly: all (any grade) toxicity was combined, instead of focusing on the important serious adverse events.
In short, this study does not gain any new insight or angle to perform more research to the first-line treatment of EGFR mutation-positive NSCLC. Hence I would suggest to

reject this manuscript for publication.

We appreciate the reviewer's analysis of the manuscript. As osimertinib is the preferred agent in the 1st line setting, to compare use to afatinib a retrospective study would be the most appropriate approach at this time. It is unfortunate that the afatinib group was not more robust, and in an effort to control for selection bias, the osimertinib group was matched to the afatinib group. As this was a retrospective chart review study, we are limited in our ability to clearly differentiate grades of toxicity as in clinical practice clinicians do not routinely grade adverse events using CTCAE. We respect the reviewer's opinion but feel that our study does offer new and additional insight into a topic which is not fully elucidated.