Peer Review File
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Reviewer A

The article entitled "Locally advanced and advanced non-small cell lung cancer patients with favourable response to initial ICIs treatment can benefit from ICI rechallenge beyond initial disease progression" shows an interesting study evaluating the efficacy of ICI rechallenge in NSCLC. The study is undoubtedly of great interest to the scientific community because it evaluates a topic that is very complex in clinical practice and has more doubts than certainties. The current retreatment with ICIs is not standardised and in some cases is even thought to be harmful. Therefore, the publication of an article of these characteristics with a cohort of 224 patients is more than interesting.

In an overall review, although there are important points that could be modified in the article, I believe that the manuscript is well written, well structured and with clear and concise language. The idea and objectives of the article are clear, and the authors use a simple and well-done methodology to reach results and conclusions that hold up well. Therefore, I believe that the article does not need major changes. Some changes are necessary, but not to the fundamental parts of the article. There is one point that I think is confusing and I think the authors could change it throughout the manuscript. They talk about locally advanced and advanced NSCLC, however, for practical purposes they are metastatic and advanced NSCLC because there are no early curative local treatments and initial immunotherapy is prescribed. The authors should clarify this point throughout the article.

Reply: We feel great thanks for your professional review work on our article. We will give the following analysis: Up to one-third of newly diagnosed patients with non-small cell lung cancer (NSCLC) present with locally advanced, unresectable disease. Most patients have already lost their best chance of surgical treatment at diagnosis. In the PACIFIC trial, a randomized, controlled phase III clinical study, durvalumab as

consolidation therapy after CCRT in unresectable stage III NSCLC patients significantly improved PFS and OS (1-2). Guidelines recommend concurrent radiotherapy for unresectable stage III NSCLC, as well as durvalumab as consolidation therapy after concurrent radiotherapy. However, in the real world, many patients with unresectable stage III NSCLC choose the same treatment regimens as advanced NSCLC due to the limited efficacy of this therapy. And since we enrolled a small number of locally advanced patients (n=56) , we don't think it affected the accuracy of results.

References:

1. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after Chemoradiotherapy in Stage III Non–Small-Cell Lung Cancer. New England Journal of Medicine 2017;377:1919-29.

2. Spigel DR, Faivre-Finn C, Gray JE, et al. Five-Year Survival Outcomes From the PACIFIC Trial: Durvalumab After Chemoradiotherapy in Stage III Non–Small-Cell Lung Cancer. Journal of Clinical Oncology 2022;40:1301-11.

Comment 1: Title: I think it should be more concise and clear. Make it clear that this is a study evaluating the ICI rechallenge.

Reply 1: We sincerely thank the reviewer for carefully reading. As suggested by the reviewer,

we have modified the title “Locally advanced and advanced non-small cell lung cancer patients with favorable response to initial ICIs treatment can benefit from ICI rechallenge beyond initial disease progression” to “Efficacy and safety analysis of ICI rechallenge therapy in locally advanced and advanced non-small cell lung cancer: a retrospective study” (see Page 1, line 1-2).

Comment 2: Abstract: in methods specify the statistical analysis used.

Reply 2: We think this is an excellent suggestion. According to the reviewer's comment, we have added the statistical analysis used in methods clearly (see Page 2, line 12-13). “Survival data were estimated using the Kaplan-Meier method or Cox

survival regression model and compared using the log-rank test in overall cohort and other subgroups"

Comment 3: Abstract: in conclusions I think the last sentence (lines 33-34) should be deleted because it is confusing and replaced by a more generic one.

Reply 3: We think this is an excellent suggestion. We have deleted the confusing sentence " Local therapy during the second-line treatment can not provide additional clinical benefit in all NSCLC patients". The relevant information has been detailed in the introduction section (see Page 14, line 5-22). "Radiotherapy can enhance tumor antigen presentation and induce anti-tumor T cell responses … confirmed this idea that local therapy during second-line treatment may benefit only a small percentage of patients."

Comment 4: Introduction, line 40: give exact data (1.8 million deaths per year from lung cancer).

Reply 4: We sincerely thank the reviewer for carefully reading. As suggested by the reviewer,

we have modified the revised manuscript as advised (see Page 5, line 2-3). "Lung cancer is the most common cancer worldwide as well as the leading cause of cancer related death with an estimated 1.8 million deaths each year"

Comment 5: Methods, line 84: XXX? Specify.

Reply 5: Thank you for your suggestion. We have replaced the "XXX?" into " the Jiangsu Cancer Hospital" (see Page 6, line 22). "We retrospectively reviewed the medical records of locally advanced or advanced NSCLC patients who received treatment at the Jiangsu Cancer Hospital between January 2019 and June 2022"

Comment 6: Results, lines 140-142: this would correspond to methods.

Reply 6: We sincerely thank the reviewer for carefully reading. We have modified the revised manuscript as advised (see Page 7, line 7-9).

Comment 7: Results (safety): since table 3 and 4 make a great summary of all these data, I think this point should be more concise and summarised by the authors.

Reply 7: Thank you for your suggestion. We have revised the safety clearly (see Page 11, line 4-19). "…and no statistically significant differences in the incidence of grade

3 and 4 AEs were observed between the two groups (17.6% vs. 14.0%, P=0.550) (Table 6)”

Comment 8: References: should be revised. There are a number of studies that are not evaluated in the discussion and are missing from the bibliography. Studies such as the following should be included:

1. Feng Y, et al. Efficacy and safety of immune checkpoint inhibitor rechallenge in non-small cell lung cancer: A systematic review and meta-analysis. Thorac Cancer. 2023;14(25):2536-2547.

2. Dolladille C, et al. Immune Checkpoint Inhibitor Rechallenge After Immune-Related Adverse Events in Patients With Cancer. JAMA Oncol. 2020;6(6):865-871.

3. Plazy C, et al. Immune Checkpoint Inhibitor Rechallenge and Resumption: a Systematic Review. Curr Oncol Rep. 2022;24(9):1095-1106.

4. Xu Z, et al. Immune checkpoint inhibitor rechallenge in advanced or metastatic non-small cell lung cancer: a retrospective cohort study. J Cancer Res Clin Oncol. 2022;148(11):3081-3089.

Reply 8: Thank you for your kind reminding. We did not use the second reference you recommended above. In our study, patients who defined PD at the end of the first line immunotherapy were enrolled. The criteria for patient enrollment in that study were different from ours, so we consider it is not suitable for this study. The remaining three valuable references have been added to the revised manuscript (see Page 14, line 3-5; Page 15, line 17-22; Page 16, line 1-4; Page 16, line 12-13). Specific content is described below.

“…A systematic review of the efficacy and safety of ICI rechallenge in NSCLC also suggested that patients with good ECOG PS had better outcomes(21)”

“Another retrospective study of ICI rechallenge in 40 NSCLC patients showed longer mPFS and ORR than ours (6.8 months vs. 5.33 months; 22.5% vs. 10.3%) (30). There are several possible reasons for this discrepancy. Our study enrolled patients with unresectable stage III or IV NSCLC, while this study included early stage NSCLC patients. In addition, our study enrolled patients without driver mutations and were

directly rechallenged with ICI beyond PD after the first-line immunotherapy treatment. While this study included 17 patients who carried mutated genes and 7 patients received chemotherapy or targeted therapy prior to ICI rechallenge treatment. Although the study suggested that ICI challenge may be an option for NSCLC after progress to immunotherapy, the results of all prognostic factors were nonsignifcant. Further prospective studies with larger sample size are needed."

"Similarly, the results of a systematic review Including 2100 patients from 17 studies demonstrated the length of PFS1 was an independent prognostic factors of PFS2 (p = 0.006) (32)"

Reviewer B

The authors concluded that ICI rechallenge generally did not provide benefit after disease progression in non-small cell lung cancer patients, but there is an obvious improvement in patients who responded to previous ICI. Some issues still need to be clarified or corrected:

Comment 1: There was a significant difference in PFS2 of resistant and responder patients. In order to claim that responder status is associated with PFS2, it should be included in the univariate analysis for PFS2 and if significant, in multivariate analysis (Table 2).

Reply 1: Thank you for your kind reminding. We've added the univariate and multivariate analyses of PFS2 in ICI rechallenge group (see Page 10, line 22; Page 11, line 1-2; Page 16, line 7-8). And they were described in detail in Table 3. "As shown in Table 3, multivariate analysis of the ICI rechallenge group (n=165) showed that response to initial ICIs treatment was associated with PFS2 (P=0.015)" "Multivariate analysis of the ICI rechallenge group (n=165) also showed that response to initial ICIs treatment was an independent factor associated with PFS2 (P=0.015)"

Table 3 Univariate analysis and multivariate analysis of factors of progression-free survival 2 in ICI rechallenge group (n = 165)

Variable	Category	Univariate analysis			Multivariate analysis		
		HR	(95%	P	HR	(95%	P

		CI)		CI)	
Sex	Male vs. female	0.999 (0.636-1.570)	0.998		
Age	>60 vs.≤60	0.997 (0.698-1.425)	0.987		
Smoking status	Smoker vs. never smoker	0.888 (0.605-1.304)	0.545		
Histology	Non-adenocarcinoma vs. adenocarcinoma	1.127 (0.801-1.587)	0.492		
TNM stage	IV vs. III	1.456 (0.942-2.249)	0.091		
Combination of radiotherapy	Radiotherapy vs. no radiotherapy	0.798 (0.541-1.179)	0.257		
ECOG PS	≥1 vs. 0	2.005 (1.295-3.104)	**0.002**	2.006(1.295-3.108)	**0.002**
Response to initial ICIs treatment	Responder group vs. resistant group	0.640 (0.447-0.916)	**0.015**	0.640(0.447-0.917)	**0.015**

ECOG-PS, Eastern Cooperative Oncology Group-performance status; ICI, immune checkpoint inhibitor.

Comment 2: In the last paragraph of "Introduction", the aim should be stated more precisely. The hypothesis is based on efficacy and safety of ICI rechallenge so its assessment should be the primary aim. The phrase in lines 75 to 77 should be changed.

Reply 2: Thank you for your suggestion. We have corrected the “The aim of this retrospective study was to assess the efficacy and safety of different second-line treatment options in locally advanced or advanced NSCLC patients” into “ The aim of this retrospective study was to assess the efficacy and safety of ICI rechallenge therapy in locally advanced or advanced NSCLC patients” (see Page 6, line 14-16).

Comment 3: The manuscript is written grammatically well but there are some corrections to be made. For example, it should be as "...Clinicans are still focused..." in line 52, "...We aimed..." in line 77.

Reply 3: Thank you for your suggestion. The revision was made according to the comments (see Page 5, line 15; Page 6, line 16).

Reviewer C

Comment 1: This is a valuable report on the treatment of NSCLC patients that need additional therapy after a first round of ICI. The data indicate that the best option is continued ICI treatment in a subgroup that responded well to the initial ICI therapy. This stresses the need for a better understanding of biomarkers predicting the response to ICI in this patient group. The paper would gain value with more discussion on this point.

Reply 1: Thank you so much for your careful review. We have added relevant content to the introduction below (see Page 17, line 7-17).

Specific content is described below.

PD-L1 expression levels, tumor mutational burden (TMB), and tumor-infiltrating lymphocytes (TILs) are regarded as some of the biomarkers predicting the efficacy of ICIs treatment. PD-L1 is a surface molecule which is expressed on different types of cells. Many studies have confirmed that PD-L1 expression was a predictive biomarker for the efficacy of ICIs (33,34). In 2018, Rizvi et al. (35) observed a correlation between high TMB and clinical outcomes for patients with NSCLC on immunotherapy treatment, which confirmed that high TMB tended to predict the efficacy of immunotherapy. Tumor-infiltrating lymphocytes (TILs) play a key role in the immunogenic reaction against tumors, and several studies supported TILs as a

prognostic and predictive biomarker (36,37). However, the identification and testing of robust and reliable predictive biomarkers are not sufficiently precise. Due to insufficient information on such biomarkers in patients enrolled in our study, we did not explore meaningful biomarkers in patients who responded well to initial ICIs treatment.

Reviewer D

This paper reports that "ICI rechallenge therapy after disease progression on initial ICI treatment did not improve clinical outcomes in patients with NSCLC; there was no significant difference in ORR between the ICI rechallenge group and the non-rechallenge group. However, patients who responded to initial ICI therapy had a better ORR and mPFS2 than those in the resistant group and may benefit from ICI rechallenge after initial disease progression" .

The paper is very interesting because of the detailed analyses within limited factors.

As mentioned in this paper, a more detailed analysis including factors such as TPS is needed.

Comment 1: Now, based on these results, would you actually re-challenge ICI if PFS1 was 6 months or longer at your institution or would you use chemotherapy or Beva?

What is your rationale?

Reply 1: Thank you for your comment. Firstly, our study suggested that patients with favorable response to initial ICIs treatment can benefit from ICI rechallenge therapy with a manageable safety profile. We would recommend ICI rechallenge therapy for patients with PFS1 of longer than 6 months if there is no better treatment option available. Secondly, whether patients will undergo ICI rechallenge therapy should also be based on their own situation, such as their physical condition, compliance, economic status, as well as their own wishes. Moreover, if patients discontinued ICIs due to severe adverse effects, it is necessary to reassess whether the adverse effects of

ICI rechallenge can be tolerated. In summary, multiple factors need to be taken into account to determine the optimal choice.

Comment 2: It is conceivable that resistance mechanisms such as downregulation of PD-L1 or MHC class I are activated by long-term use of ICI, but is there any mechanism in the literature that prevents ICI resistance or resets the immune status of tumors that have become resistant in the first ICI response group? What is the possible mechanism?

Reply 2: Thank you so much for your careful review. We will give the following analysis: CTLA-4 is a negative regulator of T-cell immune function as it stops potentially autoreactive T cells at the initial stage of naive T-cell activation. Other co-inhibitory molecules include LAG3, TIGIT, TIM3 an so on. They are all able to suppress T-cell activation and cytokine secretion, they are commonly found upregulated in tumour specimens. The common rationale for targeting co-stimulatory molecules is enhancing T-cell and NK-cell antitumour activity through agonist antibodies binding to such molecules, thus conveying activating signals to the immune cell. The co-stimulatory molecules include OX40, CD137, CD40 and so on. In addition, modulation of the tumor microenvironment and enhancement of T-cell priming have also been suggested as potentially effective ways to delay or prevent the occurrence of resistance to ICIs.

References: Villaruz LC, Blumenschein GR, Otterson GA, et al. Emerging therapeutic strategies for enhancing sensitivity and countering resistance to programmed cell death protein 1 or programmed death-ligand 1 inhibitors in non–small cell lung cancer. Cancer 2023;129:1319-50.