

Peer Review File

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Reviewer A

In this article the authors aimed to explore impact of metastatic series on efficacy of ICI through retrospective study and discovered that more metastatic organs indicated inferior clinical outcome and ICI combination could effectively control LM prognosis over monotherapy. It is significant for NSCLC with LM and guide the prediction of clinical prognosis. The choice of statistical method is appropriate. Some minor comments are followed.

1. Backgrounds (line 74-76) “Furthermore, combining atezolizumab (anti-PD-L1 antibody) with chemotherapy is also not an effective approach. Notably, the addition of bevacizumab to atezolizumab and chemotherapy appears to improve survival outcomes in patients with LM at baseline.” I advised that author should supply references here.

RE: Thanks for the comment. We have added the reference “Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial”. IMpower150 showed that patients with liver metastasis could benefit from ABCP (PFS: HR=0.41, 95%CI, 0.26-0.62; OS: 0.52, 95%CI, 0.22-0.82) rather than ACP (PFS: 0.81, 95%CI, 0.55-1.21, OS:0.87, 95%CI, 0.57-1.32) compared with BCP.

Change in the text: See [Line 75](#).

2. Methods (line 88) “Electronic medical record was collected” I advised that it should be corrected into “ Electronic medical records were collected”

RE: Thank you for the advice. We have changed the expression.

Change in the text: See [Line 91](#).

3. Results (line 135-137) “. 42 patients with genetic aberrations, 136 of which 50% (21/42) harbored KRAS mutation and 33.3% (14/42) with EGFR mutation 137 (Supplementary Fig.1)” About the aberrations of genes, the testing methods or experiments should be supplied clearly.

RE: Thanks for the comment. We have added the testing methods “detection of oncogenic driver mutations” in the article.

Change in the text: See [Line 111](#), [Line 129-132](#).

4. Results (line 152-154) “Younger patients (age<65) could get 153 OS (univariate: HR=1.526, p=0.060; multivariate: HR=1.593, p=0.050) instead of PFS benefit from 154 immunotherapy.” The P-value of multivariate was just 0.05 and according to the method the benefit should be considered

without statistical significance.

RE: Thanks for the advice. The p value was just 0.05 as mentioned. It should not be considered with statistical significance because it equaled to 0.05. But, after deliberate consideration, we thought that it had marginally significant effects and should be at least as the evidence for the hypotheses to be discussed in the “Discussion” part[1]. So we changed the expression “Younger patients (age<65) could possibly get OS (univariate: HR=1.526, p=0.060; multivariate: HR=1.593, p=0.050) benefit with **marginally statistical significance** instead of PFS benefit from immunotherapy.

Change in the text: See [Line 196-198](#).

[1]. Pritschet, L.;Powell, D.;Horne, Z. Marginally Significant Effects as Evidence for Hypotheses: Changing Attitudes Over Four Decades. Psychol Sci 2016, 27, 1036-42.

5. Discussion (line 253) “Previous studies demonstrated that liver was associated with poor PFS in patients treated with nivolumab and pembrolizumab(17, 18).” Please check the sentence and should the word “liver” be LM?

RE: Thanks for the comment. We have changed the “liver” to “LM”

Change in the text: See [Line 313](#).

6. Discussion (line 285) “Totally, 25-30% of NSCLC patients were present with BM at initial diagnosis.” I advised that author should supply references.

RE: Thanks for the advice. We have added the reference as followings in the article:

[29]Barnholtz-Sloan, J.S.;Sloan, A.E.;Davis, F.G.;Vigneau, F.D.;Lai, P.;Sawaya, R.E. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. J Clin Oncol 2004, 22, 2865-72.

[30] Mujoomdar, A.;Austin, J.H.;Malhotra, R.;Powell, C.A.;Pearson, G.D.;Shiau, M.C.;Raftopoulos, H. Clinical predictors of metastatic disease to the brain from non-small cell lung carcinoma: primary tumor size, cell type, and lymph node metastases. Radiology 2007, 242, 882-8.

Change in the text: See [Line 367](#).

Reviewer B

This is a well-written manuscript with relatively high clinical value. However, there are several concerns which should be addressed.

1. Clear inclusion and exclusion criteria should be addressed in “Patients”. A clear patient selection flow chart may be helpful.

RE: Thanks for the comment. We have added a flow chart with clear screening process of patients as Supplementary Figure 1. Additionally, we have modified the text as “As shown in Supplementary Figure 1, patients who were diagnosed as advanced NSCLC between January 2016 and February 2019 and received ICIs during the treatment course with complete medical record (baseline

radiological data and available follow-up) in Shanghai Pulmonary Hospital were enrolled in this retrospective study.” See [Line 87-90](#).

Change in the text: See [Line 87-90](#). Since we have added an additional figure, so the number of the supplementary figure has changed. See [Line 152, 206, 253 and 309](#). Additional figure legend was also added, see [Line 504](#).

2. About half of the included patients received other therapies and 33 patients underwent the surgery. So, I am concerned that the treatment strategy plays a big impact on the results.

RE: Thanks for the comment. In terms of treatment strategy, 128 patients received ICI monotherapy and 104 patients received combination therapy based on ICI. In terms of staging, 33 patients were post-surgery recurrence whereas 199 patients with stage IIIB-IV. Therefore, we performed the univariate and multivariate analysis to determine whether these parameters could have impact on PFS and OS as shown in Table 2. Pre-operation had no impact on PFS and OS. However, the treatment strategy (monotherapy or combination therapy) did influence the PFS. To exclude the treatment strategy as the confounding factor, we examined the relationship between different metastatic site and PFS, OS in monotherapy group and combination group, respectively as shown in Supplementary Figure 3-4. In analysis of PFS, the presence of LM was associated with shortened PFS in both monotherapy and combination group (mPFS: monotherapy group: 2.0 vs. 3.2 months, $p=0.010$; combination group: 3.2 vs. 7.0 months, $p<0.001$) compared to patients without LM. For BM, patients with BM had shorter PFS in monotherapy-treated group than those without (mPFS: monotherapy cohort: 1.7 vs. 3.3 months, $p<0.001$) and had inferior OS in combination group (mOS: 13.7 vs. NA, $p=0.028$). Details were described in the text.

See [Line 203-220 and Supplementary Figure 3-4](#).

3. In my opinion, the most important conclusion of this manuscript is that **LM is a negative predictive factor** for patients treated with ICIs. So, the authors should explain more in the discussion why this happens. However, the number of patients with LM is too small.

RE: Thanks for the advice. We have explained more in the discussion about why LM is a negative predictor and added the corresponding references. As shown in the text: “LM was a negative prognostic factor in NSCLC patients. A population-based data on metastatic site performed by Riihimaki et al reported that LM was associated with worst survival with 3 months of OS[3]. Previous studies demonstrated that LM was associated with poor PFS in patients treated with nivolumab and pembrolizumab[18, 19]. Reduced response in patients with LM could have several explanations. 1) Owing to liver-induced peripheral tolerance effect to maintain local immune microenvironment[20], patients with LM was not benefit population in immunotherapy setting. Liver-induced tolerance was firstly described in liver transplantation with the phenomenon that liver allografts were often without the need of histocompatibility[21]. Previous studies pointed out several mechanisms could account for liver-induced tolerance, including incomplete activation of CD8+T cells, passive or active mechanisms trap activated CD8+T cells and activation of regulatory T cells[22, 23]. 2) LM from lung cancer was reported to respond to treatment in a more similar way to liver cancer than lung cancer, in other word, in a way of tissue-specific immuno-regulation. Therefore, a hypoxia tumor condition in liver tumor microenvironment with high VEGF expression could contribute to the induction of immunosuppressive immune cells to form an immunosuppressive TME[24, 25]. 3) Reduced marginal CD8+T cell infiltration was observed in liver [19]. Poor TILs

infiltration led to the ineffective response to immunotherapy. In our study, we classified TME into four subtypes in a cohort of 46 patients with available tumor specimen. Patients with LM tended to have lower proportions of Type I tumors which are regarded as likely to benefit from single-agent PD-1/PD-L1 blockade, compared with those without LM[13].”

Additionally, the proportion of LM in our study was similar to previous studies ranging from 14.1%-20.6%. Although the sample size (patients with LM) is small, the conclusion drawn from this study is innovative with prompts for further large-cohort investigations on patients with LM .

Changes in the text: See [Line 314-332](#).

4. Some grammar errors in the manuscript should be corrected.

RE: Thanks for the comment. We have tried our best to improve the English spelling and grammar and hope it meet the requirement. See details in the article.