Peer Review File

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Reviewer A

Comment: I would like to commend the authors for a very high-quality manuscript that advances the knowledge in plausible effective methods of treatment od NSCLC patients with bone metastasis. I would highly recommend the publication of the manuscript. The authors have conducted a detailed, and highly methodological retrospective study of the NSCLC patients with and without bone metastasis prior to immunotherapy (including combination therapies- chemo and radiation) to explore the pattern of relapse, disease progression, efficacy of combination therapy in a carefully selected cohort of 544 NSCLS patients that received PD-1/PD-L1 immunotherapy with or without a combination therapy.

Strength of this study is the methodology, particularly the selection of patient cohort based on number of clinically significant inclusion criteria and use of propensity score matching at 1:1 ratio to group the NSCLC patients with similar demographics and disease characteristics according to bone metastasis status prior to immunotherapy. As authors rightly mentioned, it reduces the selection bias in a retrospective non-randomised study adding substantial credibility on findings of this study and motivation for future research. The key findings of this study highlight that patient with bone metastasis prior to immunotherapy have poor prognosis and higher disease progression. The combination therapies including localised treatment techniques such as radiotherapy did not show improved outcomes for these patients, however this finding was biased by small cohort of patients that received ablative radiotherapy, which is proving to be an effective localised treatment of NSCLC patients with bone metastasis. The key take home message from retrospective study presented in this manuscript is the future research in investigate the efficacy of stereotactic ablative radiotherapy in combination with immunotherapy for NSCLC patients with bone metastasis. Understanding of the biological mechanism resulting in poor prognosis of patients with bone metastasis is another important point highlighted by the authors.

In summary, it is a very well-written scientific paper, where authors have successfully emphasised the relevance of the work in the introduction; methodology and data analysis are systematic and appropriate for the study conducted. Appropriate statistical tools and metrices are being used. STROBE checklist has been provided and items included appropriately.

Tables and figures are self-descriptive in most cases and compliment the results and discussion. The abstract and conclusion sections are consistent with the findings of

this study.
I recommend the publication of the manuscript.

Reply: First and foremost, I would like to express my sincere gratitude for the time and effort you have devoted to reviewing my paper. Your expert feedback has been crucial in enhancing the quality of the manuscript.
My paper investigates the survival, recurrence patterns, and efficacy of immunotherapy combined with radiotherapy in non-small cell lung cancer (NSCLC) patients with bone metastases receiving PD-1/PD-L1 treatment. It stratifies patients based on the presence of bone metastases before immune checkpoint inhibition (ICI) and employs propensity score matching (PSM) to analyze advanced NSCLC patients. This method creates two matched cohorts with balanced baseline demographics and disease characteristics, allowing for a detailed examination of NSCLC patients with bone metastases undergoing immunotherapy. The novelty of our study lies in using a PSM 1:1 matching analysis to reduce selection bias inherent in retrospective non-randomized studies. Additionally, our findings indicate that patients with pre-existing bone metastases before immunotherapy have poorer prognoses and higher disease progression rates. I acknowledge that despite revisions, there are still areas needing improvement. Future research will focus on exploring the efficacy of stereotactic body radiotherapy combined with immunotherapy for advanced NSCLC bone metastasis patients and investigating the biological mechanisms leading to poor prognosis in these patients, with the goal of making a more substantial contribution to the field.
Thank you once again for your valuable insights and support. I look forward to further academic exchanges and collaborations with you.

Reviewer B

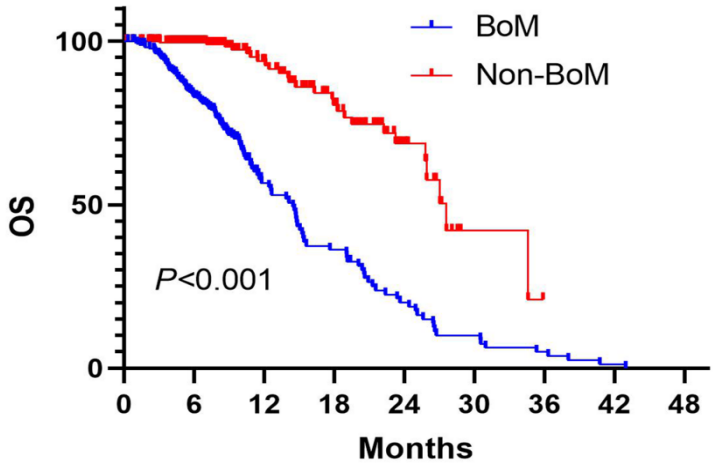
The authors retrospectively analyzed the data of advanced NSCLC patients included in a prospective observational study. They investigated the influence of bone metastasis on the ICB efficacy and the effect of adding radiation therapy to ICB in bone metastatic patients.
I agree with the authors that bone metastasis is a unique phenomenon, which could be biologically and clinically distinct from other types of metastasis and this study is of scientific interest.
I would make comments below for more scientific accuracy. I hope these comments will help improve the quality of the manuscript.

Comment 1: Please clarify the criteria for the radiologists to diagnose bone progression instead of pseudoprogression. Assessing response and progression with immunotherapy is not easy. Pseudoprogression in the bone is not rare with immunotherapy and many cases with pseudoprogressions are misdiagnosed as progression, which could be the confounding factor for worse response rate and shorter PFS in this cohort. I am curious about the overall survival difference between the two cohorts.

Reply 1: Thank you so much for your comments. First of all, we agree with you that pseudoprogression in the bone can occur during immunotherapy, but the incidence is relatively low, typically ranging from 2% to 20% (1,2) .For example, a retrospective study conducted at a single center enrolling 192 patients with bone metastatic cancer undergoing ICI therapy. It found that after 3 months of treatment, half of the patients with assessable imaging and follow-up showed bone responses, with up to 20% experiencing pseudo-progression of bone lesions (1) . In another multicenter retrospective study of 613 non-small cell lung cancer (NSCLC) patients who had previously received nivolumab monotherapy, only 3% experienced pseudoprogression after starting nivolumab treatment (2) .A third study examined 228 patients undergoing continuous treatment with anti-PD-1 therapy for three years. It found a 2% occurrence of radiological pseudo-progression, aligning with rates reported in anti-PD-1 therapy for NSCLC (3) .The seemingly higher incidence of bone pseudo-progression in the first study (20%) than that of the second and third study (2%-3%), may be related to different evaluation criteria for pseudo-progression. Assessments of tumor response by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (RECIST 1.1), the unidimensional immune-related response criteria (iRRC), and the iRECIST criteria for all patients with advanced NSCLC who received immunotherapy were performed and compared. In the current study, disease progression in the bone was determined by multiple imaging techniques, such as CT, MRT, whole body bone scan and PET/CT. Moreover, for lesions with ambiguous radiographic findings, tumor biopsies were performed and the differential diagnosis of progressive disease in the bone was determined after comprehensive review of all available clinical data, such as symptoms, lab examinations, disease status of extra-skeletal tumor lesions and disease evolution. Hence, most of the progressive status of the current study should be accurate and reliable. Nevertheless, due to the nature of observational study, we could not rule out the possibility that a minority of patients with progressive disease may still be misdiagnosed and the conclusions of the current study needed to be further validated.

Meanwhile, the results of overall survival were added in the revised manuscript. In fact, the median OS for the entire cohort was 20.4 months (95% CI:17.6-23.1). The median OS of patients with baseline bone metastases was 14.5 months (95%

confidence interval: 12.6-16.4), while the median OS of patients without baseline bone metastases was 27.6 months (95% confidence interval:25.1-30.1) (Figure 2B, shown in the following)



Number at risk

BoM	272	207	48	31	17	8	4	1	0
Non-BoM	272	204	80	45	18	3	1	0	0

Figure 2B Kaplan–Meier plots of OS in patients with BoM and Non-BoM

Changes in the text1: We added some data on the overall survival of two groups of patients. (see Page 4, line 149).

Comment 2: I do not think result 3.4 demonstrates enough data to assess the clinical value of bone radiotherapy. Patients to receive radiation for bone metastasis tend to have high risk features including intractable bone pain, pathologic fracture, high risk bone lesions, which is also associated with poor prognosis. This analysis failed to adjust these important confounders, so it is a little logical jump to discuss the efficacy of bone radiation based on this data.

Reply 2: Thanks for your comments. In the era of immunotherapy, the prognosis for patients with bone metastases is unfavorable. For example, a retrospective study of 330 patients with non-small cell lung cancer who underwent treatment with immune checkpoint inhibitors (ICIs) indicated that individuals having baseline bone metastases experienced shorter survival times than those without (median OS 5.9 months, 95% CI 4.2-7.8 versus 13.4 months, 95% CI 10.8-17.0; P<0.001) (4) .which was consistent with our findings. Hence, novel combinational therapies are needed to be explored to improve the prognosis of patients with bone metastasis. Meanwhile, increasing evidence suggests a potential sensitizing effect of radiotherapy in

combination with immunotherapy. For instance, previous preclinical investigations and retrospective analyses have provided evidence that concurrent administration of immunotherapy and radiotherapy can enhance the radiation-induced abscopal effect (5) .However, research investigating the potential immune-sensitizing effect of radiotherapy for bone metastases is scarce. Hence, we decided to analyze the impact of bone radiotherapy on survival outcomes in the current study, but found generally negative results. This outcome may be attributed to the predominance of patients receiving palliative radiotherapy, alongside those in the treatment group having more severe baseline conditions (including a higher prevalence of symptomatic patients, weight-bearing bone metastases, and multiple bone metastases), which consequently influenced the findings. On the other hand, studies suggest that hypofractionated or SBRT (stereotactic body radiotherapy) may enhance immune-sensitization (6) .Our findings also suggest that patients treated with SBRT had numerically longer survival. However, the small sample size precluded definitive conclusions and underscores the need for further research.

Table4. Baseline characteristics of patients with bone metastases receiving bone radiation or not

Characteristic	Without bone radiotherapy (N=190)	With bone radiotherapy (N=82)	*P value*
Sex			0.0984
Female	158 (83.2%)	61(74.4%)	
Male	32(16.8%)	21(25.6%)	
Age			0.3416
≤60	67(35.3%)	34(41.5%)	
>60	123(64.7%)	48(58.5%)	
Pathological type			0.8848
Scc	55(28.9%)	25(30.5%)	
Non-scc	135(71.1%)	57(69.5%)	

Number of immunotherapy lines			0.0469
First-line treatment	98(51.6%)	31（37.8%）	
Non-first-line treatment	92(48.4%）	51（62.2%）	
Immunotherapy regimen			0.6003
Monotherapy	31（16.3%）	16（19.5%）	
Combination therapy	159（83.7%）	66（80.5%）	
Smoking history			0.3290
Yes	154（81.1%）	62(75.6%)	
No	36（18.9%）	20(24.4%)	
PD-L1			0.3337
＜1%	37（19.5%）	15(18.3%)	
≥1%	78（41.1%）	27(32.9%)	
Unknown	75（37.4%）	40(48.8%)	
ECOG			0.4990
0-1	174（91.6%）	73(89%)	
2	16（8.4%）	9(11%)	
Bone pain			0.0472
Yes	97(51.1%)	53(64.6%)	
No	93(48.9%)	29(35.4%)	
high-risk bone metastases			0.0394
Yes	94(49.5%)	52(63.4%)	
No	96(50.5%)	30(36.6%)	
Bone oligo-metastasis			0.0295
Yes	98(51.6%)	32(39%)	

No	92(48.4%)	50(61%)

Changes in the text2: we added some data on table4(see new Table4 after page 17)

Comment 3: Page 1 Line 19 - the title mentions this is a prospective study, but this lane says this is a retrospective analysis, please clarify

Reply 3: This was a post-hoc analysis using data from a prospectively maintained database, which was registered as NCT047665.

Changes in the text3: The above advises have been replied and need not be modified (see Page 1 Line 19)

Comment 4: Page 1 Line 23 - the number of PFS (months) and 95% CI does not match. PFS of 7.8 months and 95% CI 0.6 - 1.1 are not compatible with each other.

Reply 4: Page 1, line 23- PFS is 7.8 months, 95% CI modified to 7.0-8.7.

Changes in the text4: we have modified our text as advised (see Page 1, line 23)

Comment 5: Figure 2. The shape of this Kaplan-Meier curve is not typical. Based on this curve, the hazard changes significantly with time for the first few months, the events are very uncommon but around 9 months, the risk exacerbates a lot. This curve should not fit Cox proportional hazard model. It would be better for the authors to discuss why the data generated these atypical Kaplan-Meier curves.

Reply 5: K-M curves during the era of immunotherapy often exhibit atypical patterns, exemplified by those observed in the Checkmate017 and Checkmate057 studies (7) .We agree with you that the K-M curves in our study did deviate from the assumptions of the Cox proportional hazard model. Statisticians are currently exploring novel statistical methodologies, including the Landmark survival analysis method (8) ,which has yet to gain broad acceptance and application. Currently, many large phase III RCT clinical trials continue to employ this model for survival calculations. Given the preliminary nature of our study, we opted not to utilize more advanced research methodologies.

Changes in the text5: The above advises have been replied and need not be modified (see new Figure 2A and Figure 2B after page 11)

Comment 6: Table 1. The meaning of "smoken" is unclear. Is this history of smoking or active smoking?

Reply 6: The meaning of "smoken" is "smoking history"

Changes in the text6: we have modified our text as advised (see Page 14, Table 1)

Comment 7: Table 3. multiple p-values were calculated, which necessitates correction of multiple comparison, such as Bonferroni correction.
Reply 7: We will perform Bonferroni correction on multiple P-values
Changes in the text7: we have modified our text as advised (see new Table3 after page 17)

Reviewer C

Comment: This is an interesting study with reasonable statistics. I made considerable suggested revision of the manuscript (attached), it requires considerable revision including editorial editing.

Reply: We appreciate the time and effort you dedicated to reviewing our manuscript and are grateful for your professional comments and suggestions. Your feedback has significantly enhanced our work. In response to your suggestions, we have made substantial revisions to the manuscript. All changes are highlighted in the PDF with tracked changes and color-coded formatting. Additionally, we have provided detailed annotations of our revisions. Thank you once again for allowing us to resubmit the revised manuscript.

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