Peer Review File

Article Information: Available at http://dx.doi.org/10.21037/tlcr-20-754

-Reviewer A-

We would like to thank Reviewer A for her/his constructive comments on our manuscript. Below are our answers to her/his comments.

Comment #1: Prevention treatment for bone metastasis with BP is the SOC to reduce the risk of SRE regardless of KRAS mt status.

Reply #1: We agree with Reviewer A that in certain malignancies (i.e. breast cancer) the use of bisphosphonate therapy (BTx) in the adjuvant setting may prevent the appearance of bone metastases (by reducing the persistence of disseminated tumor cells) and thereby might improve the outcomes (1). In lung cancer, however, the exact role of BTx in preventing the appearance of bone metastases is largely unknown (2,3). Accordingly, in lung cancer patients, BTx is only used in patients with already diagnosed bone metastases in order to reduce the incidence of skeletal-related events (SREs) and improve pain control (as already mentioned in the "*Introduction*"; page 4; lines 80-81) (2,3). In addition, we now mention in the "*Introduction"* that BTx is administered regardless of mutational status.

Changes in the text #1: The following sentence was revised in the "*Introduction*" chapter:

-page 4, lines 80-82: To date, bisphosphonates are one of the most commonly used therapeutic agents to prevent and reduce the incidence and delay the onset of SREs in LADC patients regardless of mutational status.

Comment #2: Also, sometimes RT for prevention of bone fracture and palliation.

Reply #2: We thank the Reviewer's comment. As already mentioned in the "*Introduction*", palliative radiotherapy (RTx) remains the gold standard for stabilization of impending pathologic fractures and treatment or prevention of spinal cord compression in bone metastatic lung cancer patients (page 4; lines 87-89). Based on the Reviewer's above suggestion, we revised the "*Introduction*" concerning the indications of RTx.

Changes in the text #2: The following sentence was modified as advised:

-page 3, lines 87-89: Besides BTx, radiation therapy (RTx) is also frequently used in bone metastatic LADC for stabilization of impending pathologic fractures and treatment or prevention of spinal cord compression, pathologic fractures and bone pain.

Comment #3: Page 3 Line 106: The sentence following "We present the following article in accordance with the REMARK reporting checklist." Should be in the methods.

Reply #3: We thank the reviewer for this suggestion. Of note, however, the aforementioned statement was incorporated into the "*Introduction*" section based on the recommendation of

the Journals' Editorial Office stated in the *Guidelines for Authors*: "A statement should be included at the end of the "*Introduction*" to indicate which reporting checklist was followed".

Changes in the text #3: No changes in the text.

Comment #4: CEV is not SOC in the current treatment for NSCLC as the author stated in the Method section.

Reply #4: We thank Reviewer A for bringing this inaccuracy to our attention. Drug administration was performed in accordance with the contemporary NCCN guidelines and the Hungarian health care financial regulations. With regards to chemotherapeutic agents, all included patients were treated with platinum-based combination chemotherapy either with paclitaxel and carboplatin (PC), gemcitabine and cisplatin (GC), or etoposide and cisplatin (EC). We rewrote the "*Treatment"* subchapter of the manuscript accordingly (see Page 7, lines 152-155).

Changes in the text #4: The following sentences were revised in the "*Treatment"* subchapter:

-page 7, lines 152-153: "Drug administration was performed in accordance with contemporary NCCN guidelines and the Hungarian health care financial regulations."

-page 7, lines 153-155: "Regarding CTx, patients were treated with platinum-based combination CTx either with paclitaxel and carboplatin (PC), gemcitabine and cisplatin (GC), or etoposide and cisplatin (EC)."

Comment #5: This study is too small to conclude. Also, we have already known that Stage IV KRAS mt NSCLC and bone mets are poor prognosis. So, there is no new finding in the result of current research.

Reply #5: We thank Reviewer A for this comment. Although the final number of included patients was indeed relatively small due to our strict inclusion criteria, we believe that a smaller but strictly homogenous patient cohort allows us to draw conclusions that are more scientifically accurate than those concluded based on a heterogeneous patient cohort. The majority of lung cancer-related publications in the scientific literature investigating the prognostic and predictive relevance of KRAS mutations were performed on heterogeneous patient cohorts with regards to ethnicity, histology, tumor stage, and methodology. In contrast, in our study, we included only the histologically confirmed pure lung adenocarcinoma (LADC) patients with stage IV disease, Caucasian ethnicity, and know KRAS mutational status. In addition, we only included the at-diagnosis bone metastatic cases (without any other distant organ metastases) further reducing the final number of included patients. Of note, however, we do acknowledge that the relatively small number of included patients is a limitation of our study, and our findings needs to be validated in larger patient cohorts. We have revised the "limitations" paragraph of the "*Discussion*" section accordingly (the need for a larger patient cohort was already mentioned in page 15, lines 328-329).

We agree with the Reviewer that our previous study demonstrated that the presence of KRAS mutations is associated with significantly worse survival outcomes in bone metastatic LADC patients. Yet, limited data is available regarding the association between KRAS mutational

status and the impact of therapeutic approaches in these patients. To the best of our knowledge, our study is the first investigating the prognostic relevance of KRAS mutational status with regards to specific therapeutic approaches including BTx and RTx. Besides confirming that KRAS mutations are indeed negative prognosticators in bone metastatic LADC patients, we also demonstrate that the efficacy of the aforementioned therapeutic modalities is more pronounced in KRAS wild-type patients.

Changes in the text #5: The following sentence was inserted to the "*Discussion*" chapter:

-page 14, lines 321-325: Additionally, the final number of included patients was relatively small due to our strict inclusion criteria. Nevertheless, this approach enabled us to analyze a homogenous cohort of at-diagnosis bone metastatic lung cancer patients with the same ethnicity, histology and disease stage.

-Reviewer B-

We are pleased that Reviewer B is positive about our paper and we thank her/him for providing the below suggestions.

Comment #1: Line 116 study population; did these patients only have bone metastases or also other metastases e.g. visceral or brain?

Reply #1: We thank Reviewer B for raising this point. In the current study, we only included the at-diagnosis bone metastatic patients exclusively. Accordingly, none of the included patients presented any other distant organ metastases (e.g. visceral or brain) at the time of diagnosis. We have revised the "*Study population*" subchapter.

Changes in the text #1: We have modified our text as advised:

- page 6, lines 128-129: Of note, none of the included patients presented any other distant organ metastases at the time of diagnosis.

- please see Changes in the text #5; Reviewer A

Comment #2: Line 169: Figure 1 is not necessary, as these data are already shown in table 1.

Reply #2: We appreciate the Reviewer's suggestion. Figure 1 and Table 1 show the patients' clinicopathological data, yet both contain relevant additional information compared to each other. For instance, Figure 1C shows the proportional association directly between KRAS mutational status and the administration of BTx, which is not presented in Table 1. In contrast, Table 1 provides a detailed summary of the patients' distribution with regards to ECOG

performance status and gender which is not illustrated in Figure 1. Although we agree with the Reviewer that some data are shown both in Figure 1 and Table 1, for a better understanding, we wanted to visualize the most relevant aspects of the clinicopathological data also on a separate figure.

Changes in the text #2: No changes in the text.

Comment #3: Line 178: In contrast, patients receiving BTx were significantly more likely to have ECOG 0 and RTx (Table 1).

Line 206: Importantly, we also found that in the KRAS WT subgroup patients with BTx had significantly increased OS compared to patients without BTx (median OSs were 11 months vs. 5.2 months, respectively; p=0.032.

Comment: As more patients with a lower ECOG score received bisphosphonate therapy, it is not surprising, that those patients survived better. So, it might be a selection bias.

Reply #3: The Reviewer's comment is well taken. We agree with the Reviewer that worse ECOG performance status (PS) is associated with reduced survival outcomes when comparing patients with higher ECOG PS (i.e. 2, 3 or 4) vs. lower ECOG PS (i.e. 0 or 1) (4). Yet, in our study, only ECOG 0-1 patients were included, and typically these patients have similar survival outcomes (both subgroups are labeled as having "good" PS for clinical research purposes) (5). Of note, however, we do acknowledge that the prognostic relevance of therapeutic approaches including BTx might be influenced by various clinical variables. As already mentioned in the manuscript (Table 2), BTx did not remain an independent prognostic factor in the multivariate model. Nevertheless, according to the Reviewer's suggestion, we now mention this in the last paragraph of "*Discussion*" describing the limitations of the study.

Changes in the text #3: According to this suggestion of the Reviewer, in the revised manuscript we have included two additional sentences in the last paragraph of the "*Discussion"* which is devoted to discussing the limitations of our study:

- page 14, lines 314-318: In addition, as significantly more patients with ECOG PS score 0 received BTx (vs. ECOG 1 patients), the results of the univariate analysis with regards to the efficacy of BTx might be biased. Importantly, however, ECOG 0 and 1 patients typically have similar survival outcomes (both subgroups being labeled as having "good" PS for clinical research purposes) (reference_49).

Comment #4: If the authors claim an influence on survival by bisphosphonates and radiotherapy, the also should describe and analyze the concomitant chemotherapy given, as it also influences survival

Reply #4: We thank the Reviewer for picking this up. In accordance with the contemporary NCCN guidelines and the Hungarian health care financial regulations, patients were treated with platinum-based combination chemotherapy either with paclitaxel and carboplatin (PC), gemcitabine and cisplatin (GC), or etoposide and cisplatin (EC). As proposed by Reviewer B, we performed a univariate survival analysis comparing the survival outcomes of patients treated with PC versus other chemotherapeutic approaches (GC or EC). No significant

differences in overall survival (OS) have been observed in patients treated with PC vs. GC or EC (p=0.297, log-rank test). In addition, we also compared the patient distribution with regards to KRAS mutational status (wild-type or mutant) and administered CHT agents (PC or EC/GC) and we found no significant difference in the distribution pattern (p=0.181, Chi square test).

Changes in the text #4: A detailed description of administered chemotherapy was added to the "*Treatment*" subchapter. In addition, the results of the univariate survival analysis with regards to specific chemotherapeutic agents is now included into the "*Results*" chapter and Supplementary Figure 1.

- please see Changes in the text #4; Reviewer A

- page 9, lines 194-196: With regards to combination CTx, no significant differences in OS have been observed in patients treated with PC vs. GC or EC (p=0.297, Supplementary Figure 1).

- Supplementary Figure 1. was added to the manuscript

- page 23, lines 552-557 (Figure legends): Supplementary Figure 1. Kaplan-Meier plots for OS in patients with bone metastatic LADC according to combination CTx. The OS did not differ significantly between the patients treated with PC vs. GC or EC (median OSs were 8.1 vs. 12.4 months, respectively; p=0.297, log-rank test). BTx, bisphosphonate therapy; LADC, lung adenocarcinoma; OS, overall survival; PC, paclitaxel and carboplatin; GC, gemcitabine and cisplatin; EC, etoposide and cisplatin;

References

1. Beuzeboc P, Scholl S. Prevention of Bone Metastases in Breast Cancer Patients. Therapeutic Perspectives. J Clin Med. 2014;3(2):521-536. Published 2014 May 9. doi:10.3390/jcm3020521
2. Al Husaini H, Wheatley-Price P, Clemons M, et al. Prevention and management of bone metastases in lung cancer: a review. J Thorac Oncol. 2009 Feb;4(2):251-9. doi: 10.1097/JTO.0b013e31819518fc. PMID: 19179905.
3. Lopez-Olivo MA, Shah NA, Pratt G, et al. Bisphosphonates in the treatment of patients with lung cancer and metastatic bone disease: a systematic review and meta-analysis. Support Care Cancer. 2012;20(11):2985-2998. doi:10.1007/s00520-012-1563-z
4. Simmons CP, Koinis F, Fallon MT, et al. Prognosis in advanced lung cancer--A prospective study examining key clinicopathological factors. Lung Cancer. 2015 Jun;88(3):304-9. doi: 10.1016/j.lungcan.2015.03.020. Epub 2015 Mar 28. Erratum in: Lung Cancer. 2017 Jun;108:256. PMID: 25870155.
5. Lilenbaum RC, Cashy J, Hensing TA, et al. Prevalence of poor performance status in lung cancer patients: implications for research. J Thorac Oncol. 2008 Feb;3(2):125-9. doi: 10.1097/JTO.0b013e3181622c17. PMID: 18303431.