Peer Review File

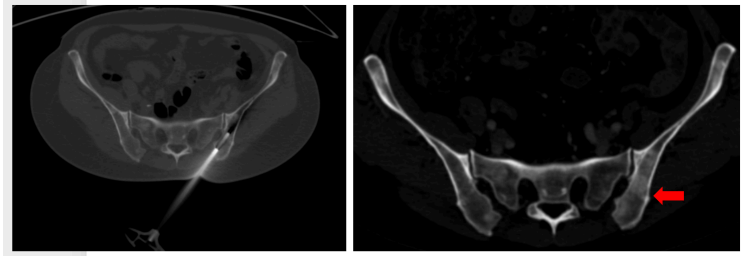
Article information: https://dx.doi.org/10.21037/tlcr-24-461

Reviewer A

The clinical case presented is very interesting and describes a rare effect of immune checkpoint inhibitor. However, several improvements are required.

• Please precise the site of bone marrow aspiration (I suppose iliacal crest..) and CT-guided biopsy (which lesion? Osteoblastic or osteolytic, which site?)

Reply： Thanks. The CT and SPECT/CT bone scan revealed that multiple bones were suffered, including multiple cervical, thoracic, lumbar, and sacral vertebrae, and the pelvis, ribs, and long bones of the limbs, and mainly were osteoblastic changes (line 106-108, page 4). The bone marrow aspiration was conducted in iliac anterior superior spine, which is the most common site. The CT-guided biopsy was conducted in iliac bone, where there was an osteoblastic lesion (see the following picture).



Changes in the text: We clarified the specific sites of bone marrow aspiration and CT-guided biopsy in line 117 and 125, page 4.

• A detailed histological description is needed, as well as histopathological images as examples.

Reply： Thank you for your advice. The bone marrow aspiration was conducted in iliac anterior superior spine. The ratio of hematopoietic tissue to adipose tissue was approximately 3-4:1. The myeloid to erythroid ratio was about 4-6:1. There was a slight increase in the number of immature granulocytes. There were 4-6 megakaryocytes per high power field (HPF). No significant morphological abnormalities were observed in the three hematopoietic lineages. Additionally, a few lymphocytes and plasma cells were observed scattered in small focal clusters. There were no tumor cells observed. The increase in the number of granulocytes was related to the use of Granulocyte Colony-Stimulating Factor after chemotherapy.

The CT-guided biopsy was conducted in iliac bone, where there was an osteoblastic lesion. Since the purpose of this bone biopsy was to determine the presence of tumor bone metastasis, the biopsy tissue was not decalcified, hence osteoclasts and osteoblasts were not clearly visible. Bone trabeculae were regularly arranged, and normal bone marrow components were visible around them. No abnormal hematopoietic or tumor cells were observed.

Changes in the text: The detailed histological description was added in the text in line 117-129, page 4. The images were added in Figure 1(B).

• In the abstract the authors mention osteoblastic bone lesions, however in the text there is no specific description of the type of lesions (osteoblastic, mixed, osteolytic). In addition, the MRI results are not totally typical for osteoblastic metastasis. Please, discuss better the results of the imaging.

Reply： Thank you very much for your reminding. The CT scan, MRI and SPECT/CT bone scan were evaluated by 2 radiologists and identified multiple bone alterations were predominantly osteoblastic lesions with minor osteolytic changes, indicating bone metastasis. Considering the osteolytic changes were minor, we described these changes as osteoblastic lesions. We had added the description of the type of lesions in the text. Bone metastases on MRI exhibit distinct imaging features depending on whether they are osteoblastic or osteolytic. Osteoblastic lesions typically appear as areas of hypointense on T1-weighted images (also known as long T1) and hypointense T2-weighted images (short T2) with minimal to moderate enhancement. Osteolytic lesions typically appear as areas of hypointense on T1-weighted images (long T1) and hyperintense on T2-weighted images (long T2) with heterogeneous enhancement. The MRI features of this patient was hypointense on T1 and T2-weighted images, which were consistent with Osteoblastic lesions. We had rewritten the phrase.

Changes in the text: We had added the description of the type of lesions in the text in line 109-113, page 4. We rephrased the “long T1, short T2” into more regular phrase “hypointense on T1 and T2-weighted images” in line 110, page 4.

• Please comment on Paget disease as a possible differential diagnosis, did you perform a radionuclide bone scan?

Reply： Thanks for your advice. First, it is truly necessary to comment on Paget’s disease of bone (PDB) as a possible differential diagnosis in this patient, because to some extent, the imaging and pathological manifestation could be similar. According to literature, PDB has several features in CT scan, like bone enlargement and deformity, "cotton wool" appearance, which were not found in this patient. In epidemiology, the incidence of PDB in Asian is much lower than in Europeans. The bone-specific ALP was normal as well.

Second, we preformed SPECT/CT bone scan, which is a kind of radionuclide bone scan using technetium-99m labeled phosphate compound. While both bone metastasis and PDB can appear on SPECT/CT bone scan, they have distinct characteristics that help in differentiating between them. Bone metastases tend to be multifocal and scattered with asymmetric distribution, while Paget's disease is more likely to show extensive involvement of multiple contiguous bones in a symmetric pattern. The uptake in Paget's disease is usually more uniform and intense over large areas, whereas metastases present as more discrete and variable in intensity. Paget's disease often causes bone enlargement and cortical thickening, which can be seen on plain X-rays or CT, whereas bone

metastases may cause bone destruction and less uniform changes. In this patient, the result of SPECT/CT bone scan did not diagnose PDB (line 106-109, page 4).

Changes in the text: We added the discussion of the PDB in line 187-193, page 6.

• How was the blood work of the patient? in particular ALP levels?

Reply： We listed the main blood tests in manuscript in line 129-143, page 4-5 and we added ALP value. The level of ALP could elevate both in PDB and bone metastasis, while in this patient, ALP was slightly increased and bone-specific ALP was normal.

Changes in the text: We added ALP value in line 139-140, page 5.

• Correct the abstract by clarifying that the patient had not symptoms related to bone disease.

Reply： Thanks for your advice. We had added this information.

Changes in the text: We clarified that the patient had no symptoms of bone disease in the abstract in line 33, Page 2.

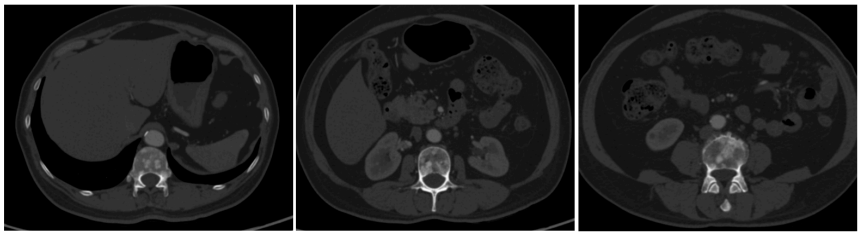
• Did the patient receive steroid for the bone lesions? If yes, how long?

Reply： No. Prednisone was administered to treat radiation pneumonitis might lead to the improvement of bone lesions (the information of dose and duration was added in manuscript). After that, we did not prescribe prednisone for her, because her bone lesions did not deteriorate in follow up and bring no symptom. We defined it as Grade 1.

Changes in the text: The information of dose and duration of prednisone was added in line 153-154, page 5.

• Did you observe any differences in the bone lesions after 6 cycles of treatment? or did they improve only after steroid for pneumonia?

Reply： Thanks. The bone lesion remained stable during the 6 cycles of treatment and did not show significant differences (the following picture). Durvalumab was suspended for four months and prednisone was administered for one month (Prednisone 30mg/day for 2 weeks, then 15mg/day for 1 week, 5mg/day for 1 week). Then the bone lesions improved (line 152-153, page5). We descripted it in the manuscript.



Oct. 2023, after 6 cycles treatment

Changes in the text: We clarified that the bone lesions remained stable in line 150-151, page5.

• Reference 12, main text, please refer to Pantano et al. (Francesco is the first name)
Reply： Thanks for your careful review. We were sorry for our mistake and had modified it.
Changes in the text: We modified Francesco to Pantano in line 217, page 7.

Reviewer B

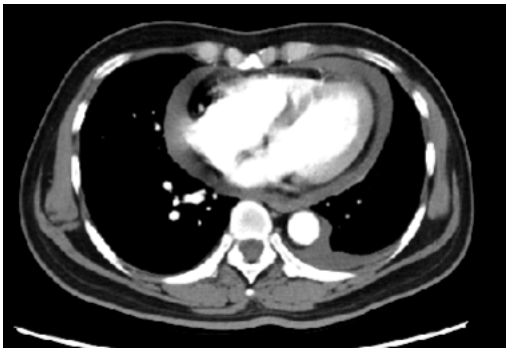
In this case report, the authors described the first case of osteoblastic bone alterations in a patient with small cell lung cancer receiving chemotherapy and durvalumab. The efforts to correctly diagnose the bone alterations detected by CT scans with the immune-related osteoblastic change should be commended. However, there are issues in this report as described below. This reviewer believes that addressing the raised concerns makes this case report much more informative and help clinicians detecting similar bone abnormalities in patients receiving immune checkpoint inhibitors.

Comments:
1. Abstract: as the grade of this osteoblastic irAE was generated based on the authors' experience, it would not be appropriate to describe it in Abstract.
Reply: Thank you. We agree with your advice and had modified in manuscript.
Changes in the text: We had deleted the "grade" in Abstract in line 23 and line 37.

2. It is surprising that this patient with small cell lung cancer had no history of smoking. Could you add information about other potential risk factors of lung cancer such as a history of passive smoking, biomass burning, and occupational exposure?
Reply: Thank you for your suggestion. We had carefully asked the patient about her potential risk factors of lung cancer. She was a food quality inspector without any exposure to asbestos, radon and other carcinogens, such as arsenic, chromium and nickel。She and her husband kept a healthy life style with no history of smoking. A report from JAMA Netw Open in 2022 revealed that the percentage of never-smokers in the SCLC population was about 16% and 31.6% were women (JAMA Netw Open. 2022;5(3): e224830.doi:10.1001/jamanetworkopen. 2022. 4830).
Changes in the text: We added more details about the potential exposure to carcinogenic factors in Case Presentation in line 86-89.

3. This patient had left cervical lymph node metastasis. Does this mean the anterior scalene lymph node metastasis? If not, the disease stage should be categorized as IVA. In addition, please specify whether the disease stage was assigned as extensive stage or limited stage.

Reply: Thanks. At your suggestion, we carefully reviewed the results of the tests of this patient. We confirmed that the patient had left supraclavicular lymph nodes metastasis and moderate pericardial effusion (the following picture). Because the pericardial effusion was moderate and the patient's family concerned about the risk of pericardiocentesis, we did not conduct pericardiocentesis. Therefore, the clinical diagnosis was stage IVa.



Changes in the text: We modified the stage in first paragraph of Case Presentation in line 86.

4. The patient was treated with etoposide (day 1–4) plus carboplatin (day 1) in combination with durvalumab. This treatment regimen appears not to be standard, as etoposide is usually administered in day 1–3. Furthermore, chemo-IO treatment was given only in the first two cycles. Finally, this patient received chest radical irradiation after completion of two cycles of chemo-IO and subsequent four cycles of durvalumab therapy. Such treatment history causes significant confusion for readers. Did this patient have limited stage small cell lung cancer, though she was treated with chemo-IO that is currently approved only for extensive stage disease instead of curative chemoradiotherapy? Instead, is this therapeutic strategy standard for extensive disease in the authors' country? The treatment shown in this report needs justification.

Reply: Yes, I totally agreed that etoposide is usually administered 100mg/m^2 per day in day 1–3. We calculated the total dose by this rule, which was 480mg. In this patient whose PS score was 1-2, we separated 480mg into 4 days to observe adverse events. Second, chemo-IO treatment was given 6 cycles. We had clarified it in line 123, page 4. After systematic, the disease was partial released and the pericardial effusion disappeared. According to NCCN guideline, patients with Extensive Stage-SCLC with good response to systematic therapy are benefit from thoracic radiation. So, this patient received thoracic radiation then.

Changes in the text: We explained in Case Presentation in line 91, page 3. The cycles of Chemo-IO were clarified in line 149, page 5 and we also modified Figure 1(A) to avoid any misunderstanding.

5. The authors speculated that prednisone administered to treat radiation pneumonitis might lead to the improvement of bone lesions. However, no information about the dose and duration of prednisone therapy is provided. I believe this information will be useful for readers.

Reply： Thanks for your advice. We had added information about the dose and duration of prednisone in manuscript.

Changes in the text: We added the information in line 153, page 5.

6. Most importantly, the authors did not provide images and detailed information regarding pathological findings of the bone lesions. Because this case report is the first to describe the osteoblastic irAE, please show the representative pathological image in main Figure and explain why the authors pathologically considered these lesions were caused by durvalumab. Was there any evidence showing the immune cell infiltration in the bone lesions compatible with typical irAE? Were there any other pathological findings?

Reply： The bone marrow aspiration showed no significant morphological abnormalities were observed in the three hematopoietic lineages. A few lymphocytes and plasma cells were observed scattered in small focal clusters. There were no tumor cells observed. A slightly increase in the number of granulocytes was related to the use of G-CSF after chemotherapy. Bone biopsy guided by a CT scan (iliac bone where there was an osteoblastic lesion) showed that bone trabeculae were regularly arranged and normal bone marrow components were visible around them. No abnormal hematopoietic or tumor cells were observed. Since the purpose of this bone biopsy was to determine the presence of tumor bone metastasis, the biopsy tissue was not decalcified, hence osteoclasts and osteoblasts were not clearly visible. The bone biopsy provided a differential diagnosis, ruling out tumor bone metastasis, multiple myeloma and other diseases of bones or blood system. But the diagnosis of bone irAE was based on comprehensive evaluation, including symptoms, history of ICI, images, pathology, blood tests and prednisone effective (see line 144, page5). There was no pathological standard to diagnose bone irAE around the world.

Changes in the text: The detailed histological description was added in the text in line 117-129, page 4. The images were added in Figure 1.

7. Discussion (lines 171–172): is there any literature supporting the claim that “when ICIs mainly affect osteoclasts, they promote osteoclasts activation, resulting in osteolytic changes”?

Reply： There were few researches about how ICIs affect bone metabolism. But the statement of “promote osteoclasts activation” was not accurate and we had modified into “promote osteoclasts formation”, based on the main references. Gresisen et al. reported that osteoclast formation was increased in PD-1 and PD-L1 KO mice (Reference 13). Moseley et al. reported a case series of bone resorption and discussed the mechanism (Reference 7).

Changes in the text: We added details about the mechanism of osteoclastogenesis in line 220-224, page 7 and modified the sentence in line 235, page 7.

8. Although the SPECT/CT bone scan was used in this report, I wonder if FDG-PT/CT could

provide any information to discriminate osteoblastic irAE and osteoblastic bone metastasis. Please discuss this point.

Reply： Thanks. Yes, PET/CT has higher sensitivity and specificity for bone metastasis. 18F-FDG PET/CT is more sensitive for osteolytic and bone marrow metastasis, while 18F-NaF PET/CT is more sensitive for osteoblastic metastasis. In our hospital, there is 18F-FDG PET/CT and the price for testing is very expensive. So, we conducted SPECT/CT bone scan and MRI in this patient. Tumors and inflammation can both have increased glycolysis with increased FDG uptake which may result in interpretive errors, so the usage of 18F-FDG-PET/CT in distinguishing osteoblastic irAE and osteoblastic bone metastasis should be carefully evaluated.

Changes in the text: We added the discussion of FDG-PET/CT in line 196-200, page 6.

9. Could you discuss the prevalence of osteoblastic bone metastasis in patients with small cell lung cancer?

Reply： Although few literature reported the incidence of osteoblastic bone metastasis in lung cancer, we added some in manuscript under your suggestion. Due to the huge amount of lung cancer patients, osteoblastic bone metastasis is worthy of attention.

Changes in the text: Added the discussion of the incidence of osteoblastic bone metastasis in line 174-176, page 6.

10. Is it possible to exclude the possibility that the bone lesions were caused by chemotherapy or chemo-durvalumab instead of durvalumab?

Reply： We have to say this is a hard question. We cannot completely exclude the possibility that chemotherapy was somehow associated with bone lesions. The conclusion that bone lesions were caused by ICI was speculated in several aspects. First, the usage of chemotherapy was far longer than ICI and no osteoblastic changes was reported. Second, the reported bone adverse events of chemotherapy were bone loss and fracture (Reference: Wissing, M.D. Chemotherapy- and Irradiation-Induced Bone Loss in Adults with Solid Tumors. Curr Osteoporos Rep 13, 140–145 (2015). https://doi.org/10.1007/s11914-015-0266-z). In this patient, bone mineral density examination was applied and showed normal. Third, bone metastasis lesions could present osteoblastic changes after chemotherapy or targeted therapy. But this patient did not have bone metastasis lesions before. Fourth, ICI had complex affects in almost every system and organ. Both clinical cases and basic studies found its impact on bones (Reference 7,12,13,14,15).

Changes in the text: None.