Good response of stage IV melanoma to high-dose radiation therapy combined with immunotherapy: A case report

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Abstract. Patients with advanced malignant melanoma (MM) often do not receive satisfactory treatment. The present study reports the case of a 51-year-old female patient with stage IV MM of unknown primary. After undergoing immune checkpoint inhibitor therapy, the patient received multiple doses of hypofractionated radiotherapy (HFRT) for the left inguinal lymph node and single-fraction high-dose-rate brachytherapy for the left and right lung metastases. After combination treatment, the patient experienced almost complete remission of the inguinal target area, significant relief of pain and discomfort and an improved quality of life. The time of lung radiotherapy lesion control was 8 months. Meanwhile, the observed lesions (observation lesions 1, 2, 3 and 5) adjacent to the target lesion received lower doses of scattering (0.9-1.8 Gy) and the time of control for these lung observation lesions was 9 months. In addition, restarting targeted therapy after cessation of other treatments due to myelosuppression resulted in a progression-free survival time of 6 months. Nevertheless, the patient developed new metastases in the brain and abdomen. The present case report demonstrates that high-dose radiotherapy combined with immunotherapy may be effective for local lesions and that multiple doses of HFRT may be superior to single-fraction high-dose-rate brachytherapy for certain patients. Low-dose scattering also shows improvement for local lesions. Furthermore, restarting targeted therapy may be effective in the presence of target sites. Thus, the present case report provides a possible therapeutic option for the treatment of advanced melanoma.

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Introduction

Malignant melanoma (MM) is an extremely aggressive tumor. By the time a patient is diagnosed with melanoma, it has often progressed to an intermediate to advanced stage, not amenable to surgical treatment, with a poor prognosis. Over the past 50 years, melanoma has become one of the fastest-growing malignant tumors, with an annual incidence of 3-7% globally (1,2). Most MMs have a well-defined primary site (melanoma of known primary; MKP), but there still exists a small number of MMs with an unknown primary site (melanoma of unknown primary; MUP). The incidence of the latter in patients with MM is ~3% (3). According to the National Comprehensive Cancer Network Clinical Practice Guidelines, both MUP and MKP are treated similarly (4). As a highly malignant immunogenic tumor, immunotherapy is one of the key treatment modalities for advanced melanoma, which can elicit durable responses in a subset of patients. Typically, melanoma is not highly sensitive to radiation therapy and the disease remission rate is not as high as expected (5). Radiotherapy is generally indicated for patients with inoperable advanced disease or postoperative metastatic recurrence. High-dose radiotherapy enhances the immunogenicity of tumors, whereas a combination of therapy and immune checkpoint inhibitors (ICIs) improves tumor control and induces and strengthens the antitumor immune response of the body (6). The synergistic efficacy of immunotherapy with radiotherapy (7-9) is strongly supported by several preclinical models and significant results have been achieved in treating some patients, particularly those with lung cancer and brain metastases (10-13). BRAF is the most important mutated gene in MM. In MM clinical trials, BRAF V600 inhibitors alone or in combination with MEK inhibitors have demonstrated potent antitumor effects (14,15).

In the present study, a patient with advanced recurrent refractory melanoma was observed. After undergoing ICI therapy, the patient received multiple doses of hypofractionated radiotherapy (HFRT) for the left inguinal lymph node and single-fraction high-dose-rate brachytherapy for the left and right lung metastases. As revealed by the results, high-dose radiotherapy may be effective for localized lesions, and multiple doses of HFRT may be superior to single-fraction high-dose-rate brachytherapy for certain patients. Besides, low-dose scattering also demonstrated improvement for

localized lesions. Restarting targeted therapy may be effective when the target site is present. The present case is presented following the CARE reporting checklist.

Case presentation

Patient. In December 2020, a 51-year-old woman presented to the First Affiliated Hospital of Army Medical University (Chongqing, China) with a left inguinal mass. The patient had no past medical history and denied a family history of cancer or psychosocial history. In January 2021, the patient underwent resection of the left inguinal mass at the First Affiliated Hospital of Army Medical University. Postoperative pathology demonstrated an MM (Fig. 1A and B) and immunohistochemistry showed HMB45, Melan-A, S-100 and Ki-67 (5-10%) positivity (Fig. 1C-F). The samples were also sent to the First Affiliated Hospital of Army Medical University Laboratory Center for high-throughput sequencing of 425 melanoma genes, which showed BRAF V600E mutation (+), low tumor mutational burden (TMB-L) and microsatellite stability (MSS) (Table I). No primary lesions were observed on the lower extremity skin, colonoscopy and colposcopy. The diagnosis was stage III MUP in accordance with the 8th edition of the American Joint Committee on Cancer for Cutaneous Melanoma staging system (16). The patient received eight cycles of teraplizumab [240 mg, day 1 (d1)] and temozolomide (200 mg, d1-d5) postoperatively. However, in July 2021, CT showed multiple metastases in the lungs and a diagnosis of stage IV MUP was considered. The treatment regimen was changed to dabrafenib [75 mg, twice a day (bid) orally (po)] and trametinib [2 mg, once a day (qd) po] for five cycles of treatment. After 2 months, CT showed scar formation in the left inguinal area, multiple small lymph nodes in the inguinal area bilaterally, the disappearance of inguinal lymph nodes and a significant reduction of nodules in the left and right lungs compared with the previous period (the inguinal lymph node tumor was 0 mm, and the largest metastases in the left and right lung tumors were ~3 and ~2.6 mm, respectively). However, in December 2021, imaging showed an enlarged left inguinal mass and new pulmonary nodules in both lungs (the inguinal lymph node tumor was 31 mm, and the largest metastases in the left and right lung tumors were ~10.6 and ~9.1 mm, respectively). Considering the resistance to the targeted therapy, the targeted therapy regimen was stopped and the patient received three cycles of albumin paclitaxel (400 mg, d1), carboplatin (500 mg, d1) and bevacizumab (400 mg, d2) at West China Hospital of Sichuan University (Chengdu, China). Further genotyping by high-throughput sequencing of the enlarged left inguinal mass at Huachuang Qide Medical Laboratory in West China Hospital of Sichuan University showed BRAF V600E mutation (+), TMB-L and MSS (Table II).

However, in January 2022, the patient consulted the Oncology Department of the Affiliated Hospital of Southwest Medical University (Luzhou, China) due to the persistent progression of the inguinal mass with pain and discomfort. PET/CT showed a soft tissue mass with increased glucose metabolism in the left inguinal region (diameter of ~48.9 mm) with a maximum standardized uptake value (SUVmax) of 17.5 (Fig. 2A). Multiple nodules were present in both lungs (the largest metastasis in the left lung tumor was 10.5 mm, and the largest in the right lung tumor was ~12.2 mm with a SUVmax of 2.9). After evaluation of the patient's condition in the Oncology Department, the patient underwent intensity-modulated radiation therapy for the left inguinal metastasis (3,414 cGy/7F) (Fig. 2B). Following radiotherapy for the left inguinal metastasis, the patient was treated with four cycles of pembrolizumab (200 mg, d0) and dacarbazine (385 mg, d1-5 po). During this period, owing to the increased and persistent lung metastases, the patient received single-fraction high-dose-rate brachytherapy, with a treatment dose of 49 Gy for the left lung metastases [D90 244 Gy by equivalent dose in 2-Gy fractions (EQD2)] (Fig. 3A) in March 2022 and 52 Gy for the right subpleural metastases (D90 269 Gy by EQD2) (Fig. 3B) 2 months later.

However, in April 2022, after receiving the four cycles of dacarbazine combined with pembrolizumab, the patient developed grade III myelosuppression. Thus, the dacarbazine plus pembrolizumab treatment was discontinued after comprehensive evaluation and dynamic observation. The myelosuppression recovered 1 month later. As aforementioned, genetic testing of the progressive groin mass showed that the BRAF V600E mutation was still present in December 2021 (when the patient was admitted to West China Hospital of Sichuan University); therefore, targeted therapy was initiated again in June 2022 (dabrafenib 75 mg, bid po plus trametinib 2 mg, qd po). After 2 months, CT showed a shrinkage of the bilateral pleural and lung multiple nodules (the largest metastases in the left and right lungs were ~10.7 and ~10 mm, respectively) (Fig. 3). The overall disease status was assessed as partial response (PR; a reduction of ≥30% in the sum of the largest diameters of the target lesions is considered PR). In October 2022, CT showed enlargement of the pulmonary nodules. As a result, the patient received two cycles of pembrolizumab (200 mg, d0) on top of the targeted therapy. After 1 month, CT showed an enlarged radiotherapy lesion in the lung, and the overall disease status was assessed as PR. However, in December 2022, multiple metastases appeared in the brain and the overall disease status was assessed as progressive disease (PD; an increase of at least ≥20% in the maximum diameter of the target lesion or the appearance of new lesions is considered PD). The metastases in the brain and lungs continued to grow and enlarged lymph nodes appeared in the abdominal cavity. The patient subsequently underwent palliative care at Shenzhen Luohu Hospital Group Luohu People's Hospital (Shenzhen, China) in which the brain metastases were treated with gamma knife surgery and six cycles of nivolumab (180 mg qd po). However, the patient refused other treatments and died 6 months later.

After the lung metastatic lesion was treated with single-fraction high-dose-rate brachytherapy, the time of lung radiotherapy lesion (target lesion) control was 8 months (Fig. 3). The observation lesions (observation lesions 1, 2, 3 and 5) adjacent to the target lesion received lower doses of scattering (0.9-1.8 Gy), and the time of lung observation lesion control was 9 months (except for observation lesion 4 in the right lung, which received a radiation dose of 7.8 Gy) (Figs. 3 and S1). After receiving HFRT for the left inguinal mass after immunotherapy, the radiotherapy target area continued to shrink, with a gradual relief of pain and discomfort and a significant improvement in the quality of life of the patient. In February 2023, an MRI showed no enlargement of multiple

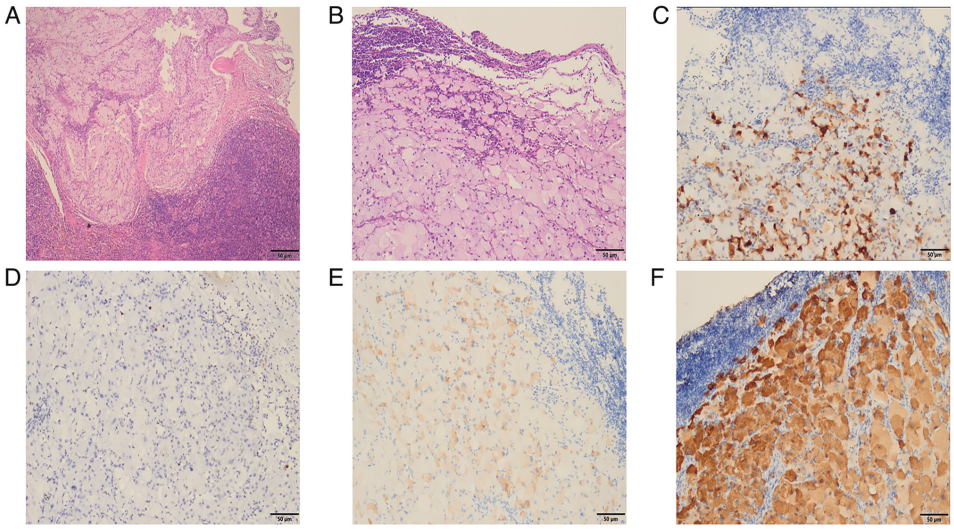

Figure 1. Pathologic and immunohistochemical images of the left inguinal mass. Hematoxylin and eosin-stained sections at magnification (A) x40 and (B) x100. (C-F) Immunohistochemistry (magnification, x100) showing positive results for the (C) HMB45, (D) Ki-67, (E) Melan-A and (F) S100 tumor markers.

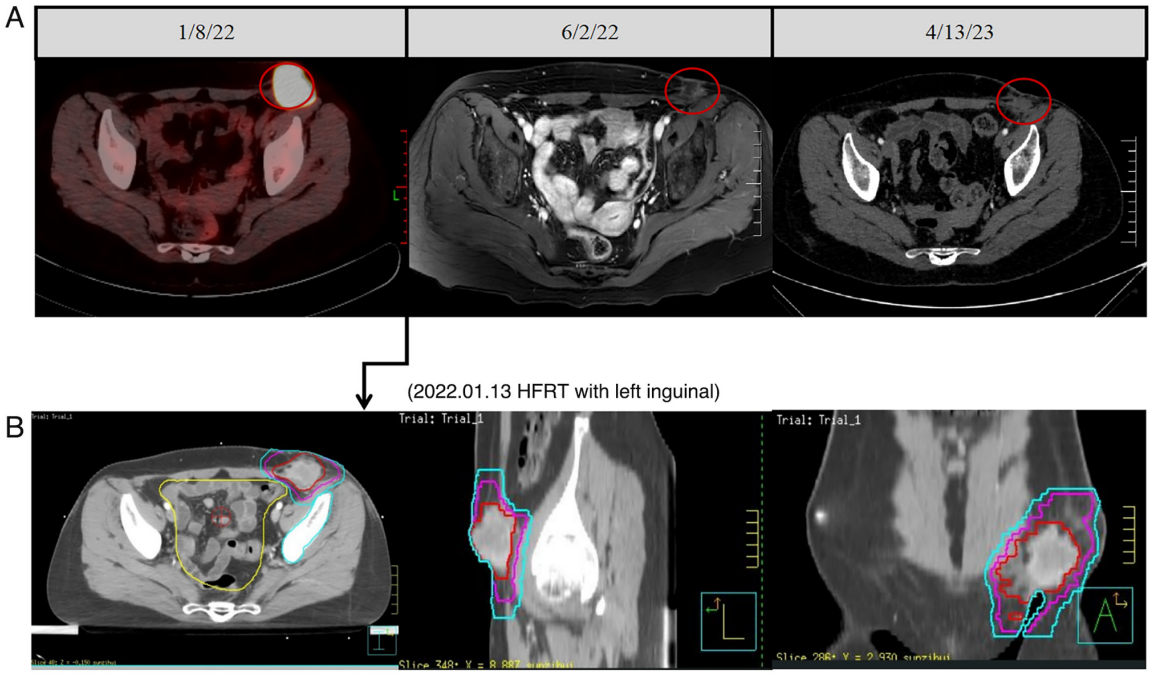

Figure 2. Treatment and images of the inguinal lymph nodes. (A) Images of the inguinal lymph nodes. (B) CT simulation images of the inguinal lymph node radiotherapy planning. The red circles are the target lesions for radiotherapy. HFRT, hypofractionated radiotherapy.

small lymph nodes in the bilateral inguinal area. The inguinal mass had largely disappeared, and the inguinal mass was assessed as complete response (CR; 100% regression of the lesion with no new lesions is considered CR) to treatment. After 2 months, the examination showed that the left inguinal mass was still under control (Fig. 2A), and the inguinal mass was still assessed as CR. The inguinal mass remained rated as CR until the death of the patient. The clinical history of the patient and the course of treatment are summarized in Fig. 4.

Pathological methods. Pathology was performed using the hematoxylin and eosin staining method. The tissues of the inguinal mass were fixed in 10% formaldehyde at 37˚C for 13 h, paraffin-embedded and sectioned to 3-4 μm. Sections were sequentially stained with hematoxylin (cat. no. BA4021; Zhuhai Baso Biotechnology Co., Ltd.) for 5-8 min and eosin (cat. no. BA4022; Zhuhai Baso Biotechnology Co., Ltd.) at 37˚C for 3-5 min. Finally, the sections were sealed and the tissues were observed by light microscope at x40 and x100 magnification (Fig. 1A and B).

Immunohistochemistry. Immunohistochemistry was performed using the Envision method. Paraffin sections were dewaxed and hydrated. Boiling antigen repair solution [0.01 M citrate buffer (pH 6.0)] was added to the sample and incubated for 3 min after the autoclave valve jetted. After

Table I. High-throughput sequencing results of 425 melanoma genes[a].

Genetic testing target	Items	Result
BRAF	V600E	Mutation
NRAS	G12D/V, G13K/R/V, Q61H/R and A146T	No mutation
MSI	/	No MSI-H (MSS)
TMB	/	6.34 Muts/Mb (TMB-L)

[a]Experiments conducted by the First Affiliated Hospital of Army Medical University Laboratory Center (Chongqing, China). MSI, microsatellite instability; MSS, microsatellite stability; TMB-L, low tumor mutational burden.

Table II. High-throughput sequencing results of 1,021 melanoma genes[a].

Genetic testing target	Items	Result
BRAF	V600E	Mutation
NRAS	Codon 12/13/59/61/117/146	No mutation
MSI	/	MSS
TMB	/	1.92 Muts/Mb (TMB-L)

[a]Experiments conducted by Huachuang Qide Medical Laboratory in West China Hospital of Sichuan University (Chengdu, China). MSI, microsatellite instability; MSS, microsatellite stability; TMB-L, low tumor mutational burden.

the repair solution was returned to room temperature, the sections were immersed in 3% methanol H_2O_2 for 10 min and washed with PBS three times for 3 min each time. The sections were then incubated with the HMB45 (undiluted; cat. no. MAB-0098; Fuzhou Maixin Biotechnology Development Co., Ltd.), MelanA (undiluted; cat. no. MAB-0275; Fuzhou Maixin Biotechnology Development Co., Ltd.), S-100 (undiluted; cat. no. Kit-0007; Fuzhou Maixin Biotechnology Development Co., Ltd.) and Ki-67 (1:500; cat. no. ZM-0167; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) primary antibodies at 37˚C for 1 h. The MaxVision TM3 HRP-Polymer (mouse/rabbit) IHC Kit (cat. no. KIT-5220; Fuzhou Maixin Biotechnology Development Co., Ltd.) secondary antibody was applied at 37˚C for 30 min. Finally, the specimens were stained with MaxVision III Ultra DAB (cat. no. KIT0038; Biotechnology Development Co., Ltd.) at 37˚C for 1 min, restained with hematoxylin (cat. no. BA4021; BaSO Biotech Co., Ltd.) at 37˚C for 2 min, dehydrated by different concentrations of ethanol and cleared with xylene. The sections were sealed and then observed under a light microscope at x100 magnification (Fig. 1C-F).

Discussion

Aggressive melanoma accounts for only 3% of all skin cancer types and is the primary cause of death related to skin cancer (17,18). Melanoma is characterized by insidious onset and easy metastasis. Patients are often at advanced stages when diagnosed. Melanoma is a highly malignant immunogenic tumor, and immunotherapy can specifically remove tiny residual tumor foci and inhibit tumor growth through various mechanisms such as by enhancing the tumor immune response of the body and interfering with tumor immune escape (19,20). Nonetheless, it was shown in a study that 25% of responders acquire drug resistance during treatment (21,22). Even the response rate of the high TMB subgroup selected according to biomarkers was not higher than 45% and remained suboptimal.

Radiation therapy is generally indicated for patients with advanced inoperable disease or postoperative metastatic recurrence. Studies have shown that radiation therapy modulates the tumor microenvironment and directly induces the death of tumor cells (23,24). Previous studies have suggested that immunotherapy combined with HFRT can achieve higher response rates and efficacy (9,25). This modality has achieved significant results in treating patients who suffer from lung cancer and brain metastases (10-13). Funck-Brentano *et al* (12) analyzed 26 patients with advanced melanoma who had consecutive anti-programmed cell death protein 1 (pd-1) monotherapy failure, and it was found that 10 patients (38%) had complete or partial remission after anti-pd-1 monoclonal antibody combined with hypofractionated radiotherapy. Additionally, Shaverdian *et al* (26) discovered that patients with advanced lung cancer who had received radiotherapy before immunotherapy had longer PFS (4.4 vs. 2.1 months) and overall survival (10.7 vs. 5.3 months) times. This combination therapy may also make radiotherapy more effective, especially by raising the incidence of 'distant effects' (27). In addition, preclinical research has shown that low-dose radiation can achieve the activation and stimulation of immune cells and the modulation of the stromal microenvironment despite not being tumoricidal *per se*, thus producing an immunotherapeutic effect (28).

In the present case report, the patient was treated with chemotherapy, immunotherapy and targeted therapy after surgery in an outside hospital. However, the results showed that the inguinal lesion progressed, multiple metastases appeared in the lungs and the combined treatment was not satisfactory. At the Affiliated Hospital of Southwest Medical University, high-dose radiation therapy was adopted, with multiple doses of HFRT for the left inguinal lymph node and single-fraction high-dose-rate brachytherapy for the left and right lung metastases, followed by repetitions of immunotherapy, chemotherapy and targeted therapy. The chemotherapy and targeted therapy regimens were identical before and after high-dose radiation therapy, and the chemotherapy combined with immunotherapy after radiotherapy resulted in myelosuppression. Immunotherapy was repeated throughout treatment. Although chemotherapy, immunotherapy and targeted therapy may have contributed to the treatment of the patient, we consider that high-dose radiation therapy based on immunotherapy had a greater role, given that the combination of high-dose radiation therapy and immunotherapy may be more

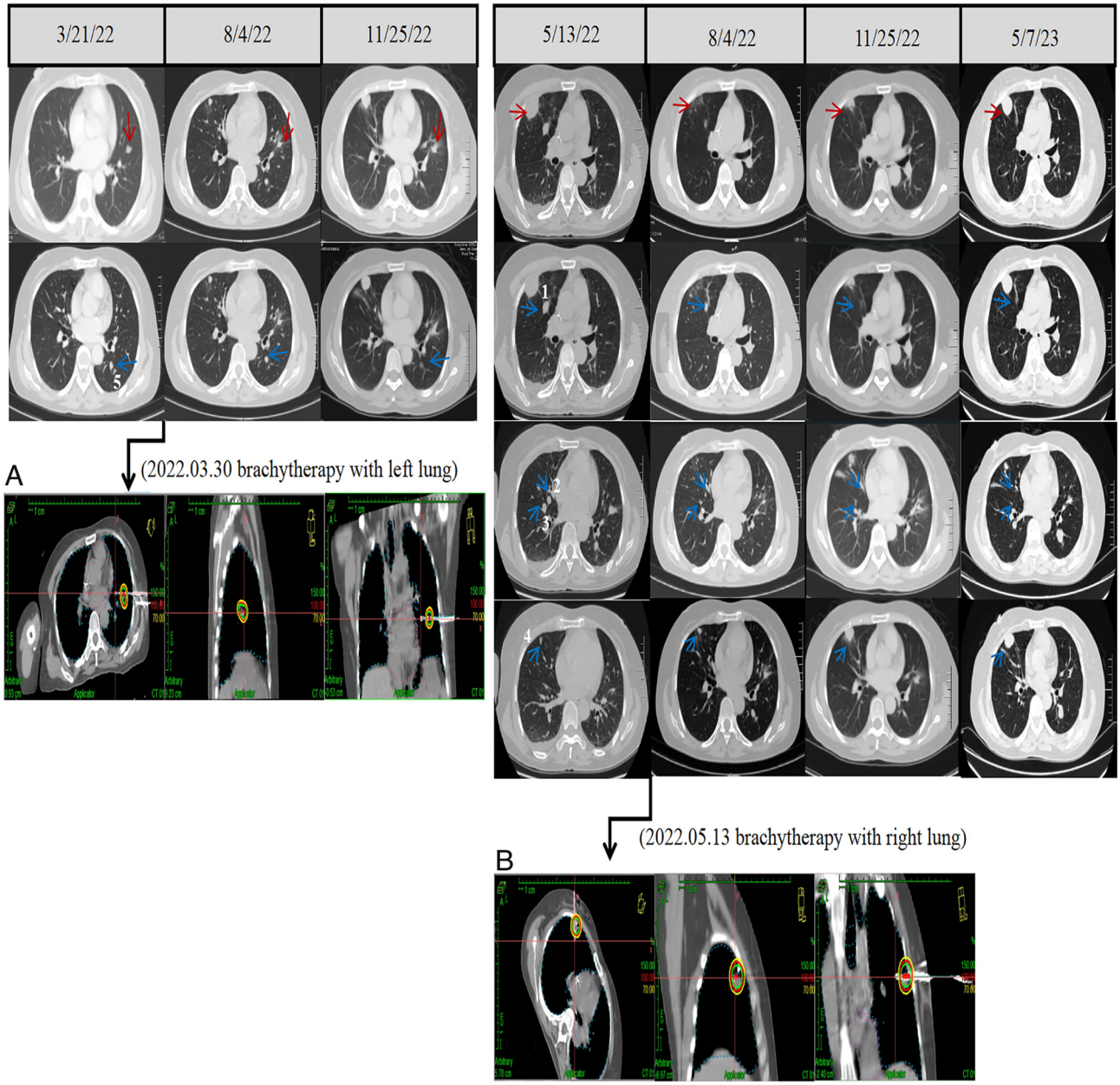

Figure 3. Treatment and images of the lung tumors. (A) CT simulation images of the left lung radiotherapy planning. (B) CT simulation images of the right lung radiotherapy planning. The red arrows indicate the target lesions for the radiotherapy and the blue arrows indicate the observation lesions receiving lower dose scattering from nearby high-dose irradiation areas. 1-5 in the image represent the five observation lesions in the lung.

effective in advanced melanoma, as indicated by the course of other treatments before and after the high-dose radiation therapy, as well as by the previously published preclinical and clinical studies (26-29). Nevertheless, more cases should be observed to reach this conclusion. Therefore, it is hypothesized that immunotherapy combined with high-dose radiotherapy may be durably effective in patients with advanced melanoma with localized lesions in the lungs and inguinal masses. This combination is similar to the study by Theelen *et al* (13). In this phase III trial, the addition of radiotherapy to immunotherapy significantly improved the response and outcomes in patients with metastatic non-small cell lung cancer. Notably, in the present case, the inguinal target area was essentially in CR until the death of the patient. The time of lung target lesion (after high-dose-rate brachytherapy) control was 8 months. The results of the radiotherapy to the lung and inguinal target lesions showed that multiple doses of HFRT may provide notably improved control of the localized lesions than single-fraction high-dose-rate brachytherapy.

In addition, the observation lesions adjacent to the target lesions received lower doses of scatter from the HFRT of the target lesion (0.9-1.8 Gy), and most of the observation lesions (observation lesions 1, 2, 3 and 5) were sustainedly controlled [except for one observation lesion (lesion 4) in the right lung, which received 7.8 Gy. It was considered that the sustained remission of the observation lesions receiving low-dose scattering was attributed to the activation of immune cells by low-dose scattering, which regulated the microenvironment and thus regulated immunotherapy. In a study by Sezen *et al* (30), a 73-year-old female patient with metastatic

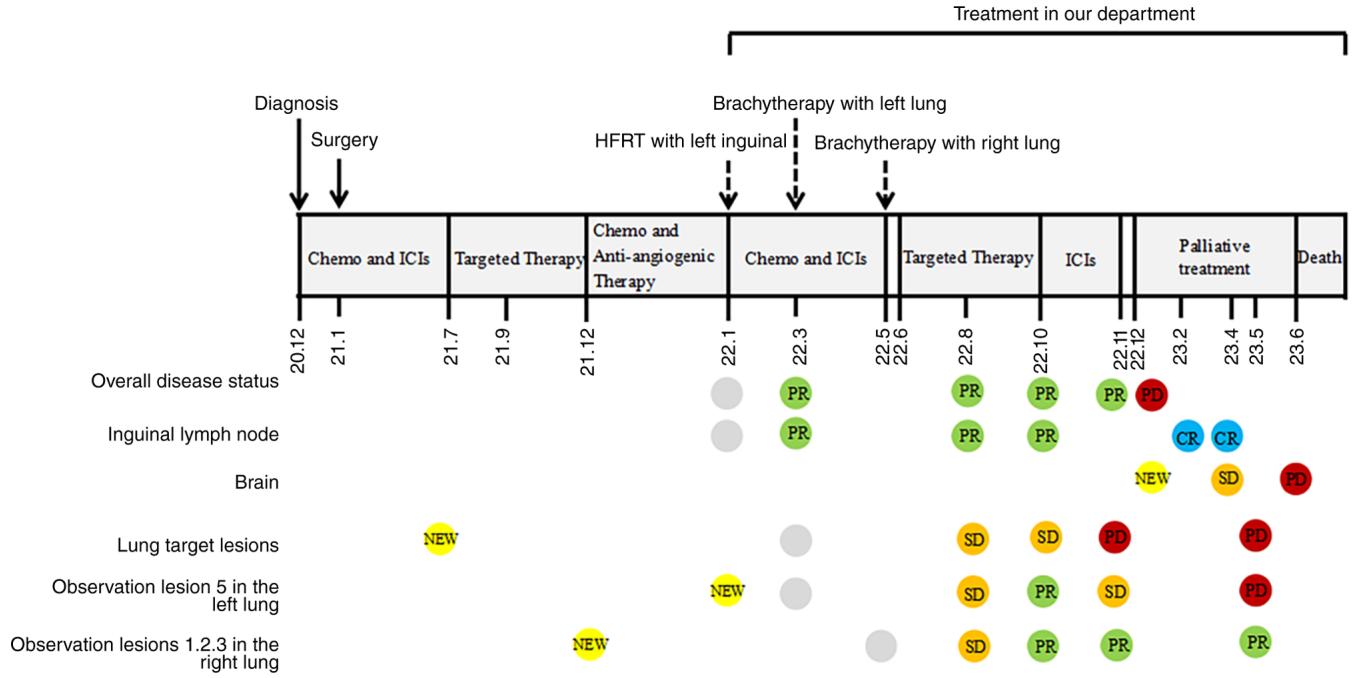

Figure 4. Timeline of the diagnosis and treatment process. New metastatic lesions are indicated in yellow and grey indicates the baseline. HFRT, hypofractionated radiotherapy; ICIs, immune checkpoint inhibitors; PR, partial response; PD, progressive disease; CR, complete response; SD, stable disease.

vaginal mucosal melanoma received treatment with ibritumomab and nivolumab. In the liver, a second metastatic lesion received lower doses of scattering from the adjacent first metastatic lesion (received HFRT). The second metastatic lesion disappeared after 24 months, which was very similar to the present study. At present, the choice of HFRT and segmentation modality remains inconclusive. In the present case, the low-dose scatter of 0.9-1.8 Gy showed a relatively good effect for the observation lesions (observation lesions 1, 2, 3 and 5), whereas a radiation dose of 7.8 Gy was ineffective for observation lesion 4, which may be attributed to the fact that the lesion received low-dose scattering.

Targeted therapy is effective in patients with advanced or metastatic tumor with well-defined target mutations. Therapy combined with BRAF and MEK inhibitors can improve therapeutic effects and postpone the onset of resistance to these agents (14,15). Lee (31) found that targeted therapy with afatinib could be restarted after the development of resistance in advanced non-small cell lung cancer and in their case the primary tumor continued to shrink after treatment. Similarly, a prospective survey found that patients with advanced melanoma with BRAF V600 mutation and disease progression after treatment with BRAF and MEK inhibitors, who were re-treated with dabrafenib after 3 months, still had objective remission and disease control rates of 32 and 72%, respectively (32). In the present study, the patient showed a significant shrinkage of pulmonary metastases after 2 months of combined dabrafenib and trametinib targeted therapy and was assessed to be PR, but resistance developed after 5 months. After the 1-month discontinuation of targeted therapy, genotyping of the enlarged left inguinal showed that the BRAF V600E mutation was still present, which supported the subsequent restart of targeted therapy. Hence, targeted therapy was restarted after 6 months of discontinuation (Fig. 4). After one cycle of restarted targeted therapy, the disease was assessed as PR, with a PFS of 5 months. This suggests that restarting targeted therapy may remain effective as long as the target is present, despite tumor heterogeneity or alteration of tumor genes after treatment disruption.

After reviewing the entire treatment course of the present patient, some limitations do exist. First, after the patient underwent resection of the left inguinal mass without searching for a primary site at an outside hospital, a dissection of the inguinal lymph nodes should have been performed, which was not the case. Second, according to the National Comprehensive Cancer Network Clinical Practice Guidelines (4), the patient should have received targeted therapy after resection of the inguinal mass due to the BRAF V600E mutation (+), and should have switched to immunotherapy if targeted therapy was ineffective; however, the patient's post-surgical treatment plan in the outside hospital was not standardized: The patient was administered an immunotherapy-combination chemotherapy regimen post-surgery and then switched to targeted therapy after the progression of the treatment. In addition, the primary site was not located from disease onset to death. The preferred treatment regimen for melanoma of the extremities, mucosa and skin varies. In the present study, since the melanoma type was not determined, the treatment of the disease was not effective.

In summary, the present report confirmed that immunotherapy combined with high-dose radiotherapy may be durable and effective for localized lesions in the lungs and inguinal masses for certain patients with advanced melanoma. The present patient had almost CR in the inguinal target area until death, and the time of lung target lesion (after high-dose-rate brachytherapy) control was 8 months. As such, it can be hypothesized that multiple doses of HFRT may be notable better than single high-dose-rate brachytherapy for certain

patients. Low-dose scattering at 0.9-1.8 Gy may modulate the tumor microenvironment and improve the immune efficacy of localized lesions. In addition, restarting targeted therapy may remain effective as long as the target is present. Certainly, this needs to be confirmed through further exploration.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XD contributed to data collection and analysis and to the writing of the manuscript. KX, XH and SC contributed to implementing the treatment and the collection of case information. QG, HW, XL and QW contributed to the data collection and analysis, and edited the research for intellectual content. HY contributed to the conception and design of the study and took full responsibility for the project, ensuring that any concerns regarding the integrity or accuracy of any portion of the project was duly looked into and addressed. XD and HY confirm the authenticity of all of the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the patient for the case information and images while the patient was alive. After the patient's death, written informed consent was obtained from the patient's family for the case information and images to be published in this case report. Additionally, the patient and the family member provided the results from the other institutions [First Affiliated Hospital of Army Medical University (Chongqing, China) and West China Hospital of Sichuan University (Chengdu, China)].

Competing interests

The authors declare they have no competing interests.

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