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Teaching Case

Overcoming Immunotherapy Resistance With Radiation Therapy and Dual Immune Checkpoint Blockade

Tara Davidson, MD,[a] Henan Zhang, MD,[b] Haidong Dong, MD, PhD,[b] Michael P. Grams, PhD,[c] Sean S. Park, MD, PhD,[c] and Yiyi Yan, MD, PhD[d,*]

[a]Departments of Internal Medicine, Mayo Clinic, Rochester, Minnesota; [b]Departments of Urology, Mayo Clinic, Rochester, Minnesota; [c]Departments of Radiation Oncology, Mayo Clinic, Rochester, Minnesota; [d]Division of Medical Oncology, Mayo Clinic, Rochester, Minnesota

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Introduction

Studies have shown radiation therapy (RT) efficacy in the treatment of melanoma dating back to the 1970s, typically used as adjuvant therapy after resection in early stage disease or as late stage palliation.[1-3] Preclinical and clinical evidence suggest triple therapy with RT and dual immune checkpoint blockade [anti−cytotoxic T-lymphocyte−associated antigen 4 (CTLA-4) + anti−programmed death 1/programmed death ligand 1 (PD-1/PD-L1)] is a complimentary technique.[4-10] We present 2 cases using the same novel regime of combination RT plus nivolumab (NIVO) and ipilimumab (IPI) to treat metastatic melanoma with extensive locoregional disease and suggest potential biomarkers for response monitoring in patients with combination RT and immunotherapy.

Case 1

A 66-year-old male with stage IIIA (T3aN2aM0; American Joint Committee on Cancer seventh edition) BRAF wildtype right thigh melanoma was initially treated with wide local excision and sentinel lymph node resection followed by complete right inguinal lymph dissection. Pathology confirmed Clark level IV, Breslow depth 3.16 mm, nonulcerated primary with 6-mm mitotic rate, absent tumor infiltrating lymphocytes, negative perineural invasion, and positive angiolymphatic invasion. Margins were negative with micrometastases in 3 of 26 lymph nodes. Fifteen months later, an asymptomatic regional recurrence in his right inguinal nodal basin was detected on a surveillance positron emission tomography (PET) scan. He was treated with a right pelvic/external iliac and obturator lymphadenectomy with 5 of 14 inguinal/external iliac, and 1 of 4 right obturator lymph nodes were involved with metastases and extranodal extension. Adjuvant radiation to the right external iliac nodal area (48 Gy in 20 fractions) was delivered without systemic therapy after his surgical intervention. Four months later, asymptomatic recurrence near the right iliacus muscle was noted and IPI (3 mg/kg every 21 days) was initiated. After 4 cycles of IPI, treatment was transitioned to pembrolizumab (PEMBRO; 200 mg every 21 days) due to disease progression, and concurrent palliative radiation 40 Gy in 10 fractions to the pelvis and 30 Gy in 10 fractions to T2 spinal metastasis was delivered. Near complete response

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Data sharing statement: The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Corresponding author: Yiyi Yan, MD, PhD.; E-mail: yan.yiyi@mayo.edu

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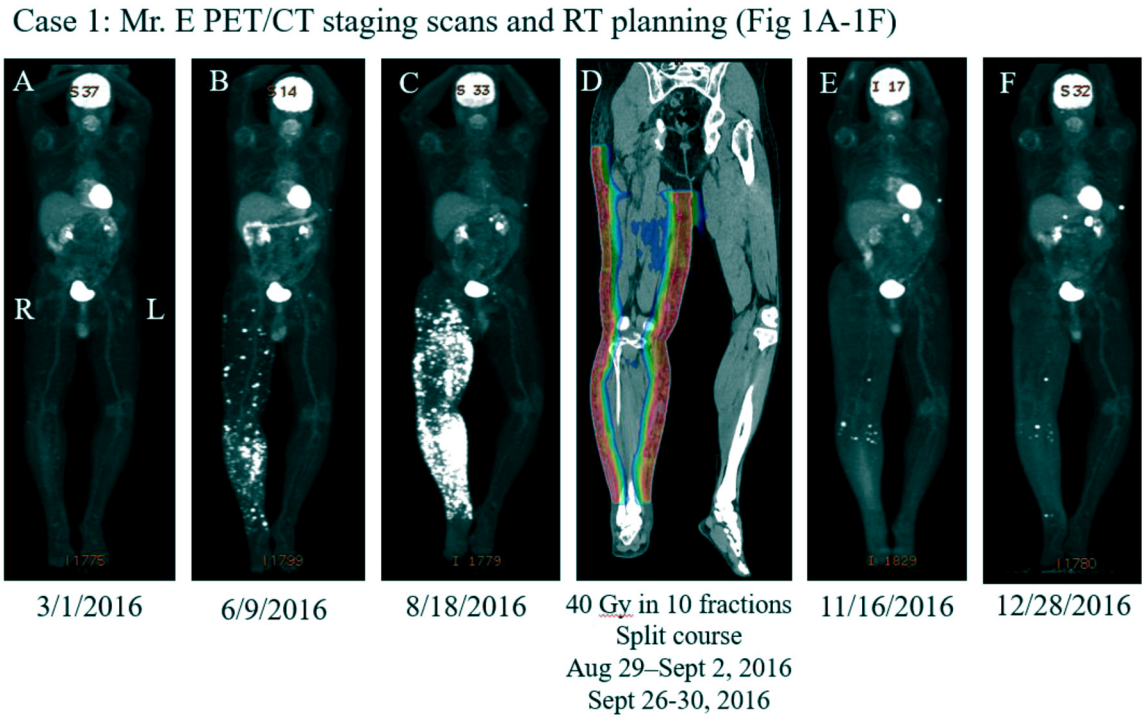

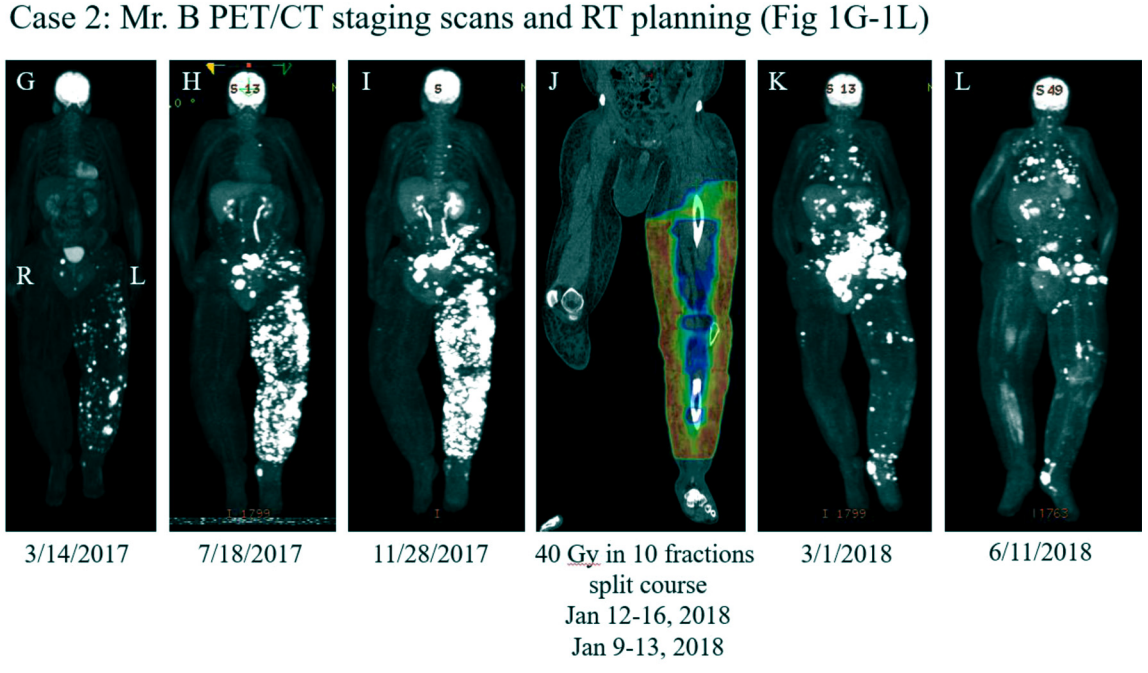

Fig. 1 Staging positron emission tomography (PET), computed tomography (CT) scans, and radiation therapy (RT) planning for both cases.

was seen after 4 cycles of PEMBRO (Fig 1A). However, after 18 months of PEMBRO, the patient developed innumerable subcutaneous soft tissue metastases throughout the right lower extremity (RLE) and new lesions in the chest wall, right lung, and left adrenal gland (Fig 1B). Paclitaxel (80 mg/m^2, d1, d8, d15, every 28 days) was added to PEMBRO, but he experienced disease progression after 2 cycles of chemo-immunotherapy (CIT) (Fig 1C). He developed severe pain, tightness, and neuropathy from the large disease burden in his RLE with

impending compartment syndrome. After multidisciplinary discussion, he was treated with a novel triple combination NIVO/IPI + RT (described in the following sections) and dramatic response was noted (Fig 1E).

Case 2

A 45-year-old man with medical history for aplastic anemia was treated with total body irradiation (TBI) and bone marrow transplant at the age of 12, post-TBI sequela including graft-versus-host skin disease and multiple non-melanoma skin cancers. (Unfortunately, the specific details of his TBI therapy were not available as this occurred >33 years prior at an outside institution.) At 41 years old, T2aN0M0 desmoplastic melanoma of his left posterior calf was diagnosed and he was treated with wide local excision. Three years later, his melanoma relapsed in multiple left calf/thigh skin and subcutaneous nodules. Biopsy confirmed BRAF wildtype melanoma and PET demonstrated extensive involvement of his left leg, bilateral inguinal basins, and scrotum (Fig 1G). PEMBRO (200 mg every 21 days) was initiated; however, disease progressed after 4 cycles (Fig 1H). The addition of carboplatin/paclitaxel (area under the concentration 5 dl/175 mg/kg every 21 days) to PEMBRO led to initial mixed response, but the disease progressed diffusely after 6 cycles of CIT combination (Fig 1I). Like case #1, this patient experienced limited mobility from severe swelling and pain in his extremity. At that point, he was also treated with the same novel NIVO/IPI + RT combination and experienced similar dramatic response (Fig 1K).

Methods and Materials

Compliance with ethical standards

Participants provided written informed consent to take part in the study. Biospecimen collection was performed under the research protocol approved by the Mayo Clinic Institutional Review Board Committee (15-000934) in accordance with regional and national guidelines.

NIVO/IPI + RT regimen

Using volumetric modulated arc therapy (VMAT), 40 Gy was delivered in 10 fractions in a split-course to coordinate with immune checkpoint inhibitor (ICI) infusion (2 NIVO/IPI infusions 21 days apart). VMAT was used to treat the rind of soft tissue involved with melanoma while sparing the core of the extremity to reduce the risk of lymphedema and bone marrow suppression. The planning objectives were such that 95% of the target (soft tissue rind) received 40 Gy, whereas the mean dose to the core of the extremity was limited to 15 Gy. The maximum dose to the genitalia was limited to less than 10 Gy. The treatment planning system used was Varian Eclipse and dose was calculated using the Anisotropic Analytical Algorithm (version 11; Varian Medical Systems). Due to the length of the treated area, the VMAT approach required 3 separate isocenters spaced approximately 22-cm apart. The dose contribution from each isocenter overlapped and allowed for a gradual dose gradient, removing the need for sharp field matching. Each plan used 3 to 4 partial arcs per isocenter to avoid entrance dose to the contralateral leg (Fig 1D). Patient-specific quality assurance was performed using an ion chamber array, which confirmed that the dose delivered agreed with the plan. RT treatment was subsequently delivered using the 6 MV output of a TrueBeam linear accelerator (Varian Medical Systems) with image guidance. NIVO/IPI therapy was continued beyond RT per standard of care.

Flow analysis of human T-cells isolated from peripheral blood

Participants provided written informed consent to take part in the study and biomarker study was approved by the institution review board. The following panel of antibodies was used for analysis of peripheral blood mononuclear cell populations: CD8-PE-Cy7 (clone RPA-T8, catalog 304006; BD Pharmingen), CD11a-APC (clone HI111, catalog 301212; BioLegend), PD-1 FITC (clone EH12.2H7, catalog 32990; BioLegend), CX3CR1-APC/Cy7 (clone 2A9-1, catalog 341616; BioLegend), BCL-2-interacting mediator of cell death (Bim)-PE (clone C34C5, catalog 12186S; Cell Signaling Technology, Danvers, MA), Ki-67-BV421 (clone B56, catalog 562899; BD Biosciences), and Granzyme B-PerCP (clone CLB-GB11, catalog NBP1-50071PCP; Novus Biologicals, Danvers, MA). CD8+ T-cells were first stained for surface markers followed by intracellular staining. Flow cytometry data were collected on a CytoFLEX LX (Beckman Coulter, Atlanta, GA). Flow cytometry analysis was performed with FlowJo software 10.4 (Tree Star, Palo Alto, CA).

Results

Both patients tolerated the NIVO/IPI + RT treatment regime without any new onset adverse events.

Patient 1 experienced clinical and radiologic improvement in the RLE and systemically on PET scan obtained 10 weeks after RT (Fig 1E) which was noted to sustained 6 weeks later (Fig 1F). Except for worsening brain metastases, he experienced a few months of stable systemic control, then he developed diffuse progressive disease outside

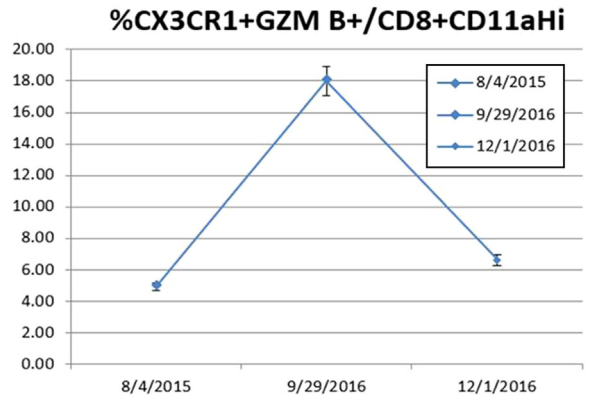

Fig. 2 CX3CR1 + CD8+ T-cell measurements for case 1 before, during, and after radiation. CX3CR1 + CD8+ T-cell level was low before radiation therapy (RT) when the patient experienced significant disease progression with chemo-immunotherapy. CX3CR1 + CD8+ T cell level increased concurrent to nivolumab/ipilimumab (IPI) + RT when he had antitumor benefit. Decreased level of CX3CR1 + CD8+ T-cells after nivolumab/IPI + RT is likely due to the tissue migration of this effector T-cell subset.

of the radiated leg. He died due to central nervous system melanoma burden 8 months after NIVO/IPI + RT treatment.

Patient 2 experienced symptomatic relief shortly after therapy began. PET scan performed 3 months after treatment showed dramatic disease response in the left lower extremity (Fig 1K). Similar to patient #1, his disease later

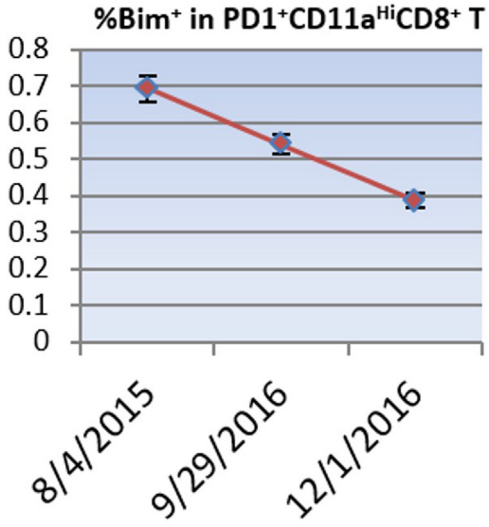

Fig. 3 Dynamic changes of BCL-2-interacting mediator of cell death (Bim) + CD8+ T-cells in peripheral blood during the course of nivolumab/ipilimumab + radiation therapy treatment. Percentage of Bim^{+} cells in $CD11a^{high}CD8+$ T-cell population isolated from the peripheral blood obtained from the same patients at same time points as in Figure 2.

progressed beyond the RT field. He received further CIT, with continued disease improvement in the left lower extremity and mixed response in other areas (Fig 1L). He also died 8 months after NIVO/IPI + RT treatment.

Peripheral blood draws were obtained to assess for potential biomarkers before, during, and after NIVO/IPI + RT therapy. Case 1 levels of CX3CR1 + CD8+ T cells (Fig 2) and Bim levels (Fig 3) are included and further described in the discussion section.

Discussion

There is renewed interest in the immunomodulatory effects of RT in the era of immunotherapy because preclinical models suggest that RT can improve efficacy of immune checkpoint blockade, and conversely ICI can increase the efficacy of RT (not only locally but systemically).[4-7] The term *abscopal effect* refers to a rare phenomenon of tumor regression at a site distant from the primary site of RT.[6] How RT exhibits abscopal effect is not fully defined but preclinical models have established that it is T-cell dependent.[6] In terms of how this might be leveraged in RT plus IPI and NIVO combination regimens, clinical models suggest that RT increases diverse T-cell activation through increased local expression of MHC class 1 molecules, which improves the antigen presenting ability of antigen-presenting cells (APCs).[6,8,9] CTLA-4 blockade suppresses T- regulatory cells, thus increasing the ratio of CD8/T-regulatory cells, and the addition of PD-1/PD- L1 inhibition increases the effector population and overall CD8+ T cells.[10] These activated CD8+ T-cells can migrate through the body and infiltrate the metastases outside of the irradiated field, causing systemic antitumor benefit.[10]

Prior clinical studies suggest that combining RT with either single-agent IPI or NIVO appears safe and promising; however, little has been reported on RT in combination with both IPI and NIVO.[11–14] In melanoma specifically,[5] a recent phase I study of NIVO/IPI + RT in patients with advanced melanoma was pursued to address the safety of this combination.[5] Although standard ICI dosing was used (NIVO 1 mg/kg and IPI 3 mg/kg every 21 days), 2 RT dose regimens were explored: cohort A received extracranial RT with a dose of 30 Gy in 10 fractions and cohort B received 27 Gy in 3 fractions. Patients responded to treatment outside of the irradiated volume (cohort A 5/10; cohort B 1/9). No patients had progression of irradiated metastases. Safety profile showed no marked difference between historic NIVO/IPI and the novel NIVO/IPI + RT.[5] RT did not compromise the ability of patients to receive their intended combination immunotherapy.[5] The trial was not designed to assess efficacy. More direct comparison studies of various RT regimens and outcomes are needed. Our patients received the same novel dosing schedule with noted dramatic, rapid, and prolonged disease control within the RT field as well as systemic benefit.

There are currently no reliable biomarkers to help identify patients who may benefit from this combination or to monitor response to therapy.[5] Previous studies have reported that the chemokine receptor CX3CR1 identifies anti-PD-1 therapy-responsive effector CD8+ T-cells in peripheral blood with lower frequency in ICI nonresponders compared with ICI responders after anti-PD-1 monotherapy.[15,16] These T-cells are able to withstand the toxicity of subsequent chemotherapy with preserved antitumor activity.[12,13] Based on these prior studies, we postulated that CX3CR1 could also be used as the potential T-cell biomarker for responsiveness to RT and immunotherapy combination treatment. To address this, we examined the frequency of CX3CR1 + CD8+ T-cells in the setting of NIVO/IPI + RT combination treatment (before, during, and after radiation) for our case 1 patient (Fig 2). CX3CR1 + CD8+ T-cell level was low before RT when our patient experienced significant disease progression after initial CIT. The level increased concurrent to RT + ICI treatment while he was experiencing significant antitumor benefit. The subsequent decrease of CX3CR1 + CD8+ T-cell level seen after NIVO/IPI + RT completion was likely due to the tissue migration of this effector T-cell subset. This suggests that the CX3CR1 + CD8+ T-cell level could be explored as a potential biomarker for RT + ICI treatment.

Bim is a proapoptotic downstream signaling molecule of the PD-1 pathway. Its detection in T-cells is significantly associated with expression of PD-1 and effector T-cell markers.[15] Previous studies have demonstrated that Bim levels decrease after successful anti-PD-1 therapy[15,17] and remain largely unchanged in patients who did not respond to PD-1 blockade. Therefore, elevated Bim levels at baseline that decline can serve as a positive treatment prognostic marker. Subsequent decrease in Bim levels after initiation ICI can be predictive of ICI response. The level remains unchanged after successful CIT combination.[17] Interestingly, for case #1, the Bim level decreased during the course of NIVO/IPI + RT treatment (Fig 3), concurrent with radiologic response. Bim levels could also be further explored as a potential biomarker for NIVO/IPI + RT.

RT and immune checkpoint inhibition is not a novel approach; however, we are continuing to learn more about how triple therapy (RT + IPI/NIVO) affects both the tumor microenvironment and the systemic tumor burden. For our 2 cases we were able to alleviate significant side effects and avoid the debility of surgery. Although our treatment strategy needs to be validated in a prospective study, given the response and safety we observed here, this approach can be used in cases when a typical RT approach is not feasible and patients are not eligible for trials. Additionally, CX3CR1 + CD8+ T-cell and Bim levels can be obtained with simple peripheral blood draw and could be used as potential biomarkers for response prediction and monitoring in patients receiving ICI/RT combination treatment.

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