case reports

Soft Tissue Leiomyosarcoma With Microsatellite Instability, High Tumor Mutational Burden, and Programmed Death Ligand-1 Expression Showing Pathologic Complete Response to Pembrolizumab: A Case Report

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Introduction

Locally advanced/metastatic sarcomas generally have a poor prognosis with limited therapeutic options. Pembrolizumab has been shown to be efficacious in solid tumors with deficient mismatch repair (MMRd) or high level of microsatellite instability (MSI-H).[1-3] Response to pembrolizumab was also found to be associated with a high tumor mutational burden (TMB) of 10 mutations/megabase (Mb) or more in solid tumors.[4] Sarcomas are, however, severely under-represented in the studies that yielded these findings. Programmed death ligand-1 (PD-L1) protein expression has also been included as an US Food and Drug Administration–approved predictive biomarker for programmed cell death protein 1 (PD-1) inhibitor therapy in various carcinomas but not for sarcomas, with the thresholds of PD-L1 expression predicting response to PD-1 inhibitor therapy in sarcomas still being undefined. There are also little data on how MSI-H status correlates with TMB and PD-L1 expression in sarcomas and to what extent each biomarker predicts response to PD-1 inhibitor therapy. In this case report, we describe a metastatic leiomyosarcoma with MSI-H status, high TMB (> 10 mutations/Mb), and significant PD-L1 expression, showing pathologic complete response to pembrolizumab, a PD-1 inhibitor.

Case Presentation

The patient is a 54-year-old Malay male who first presented with an enlarging abdominal wall mass. Computed tomography imaging revealed a heterogeneous soft tissue mass centered in the left rectus muscle measuring 7.0 × 5.2 × 8.8 cm with no distant metastases. Open excision of the tumor was performed, with clear resection margins of 2-3 mm achieved.

This study was approved by a centralized institutional ethics review board. Informed consent was obtained from the patient for the tests performed.

Histologic examination of the tumor showed a neoplasm composed of fascicles of malignant spindle cells, with eosinophilic cytoplasm (Fig 1) showing multifocal positivity for desmin and caldesmon on immunohistochemistry (IHC), in keeping with a leiomyosarcoma, FNCLCC grade 3. Fluorescence in situ hybridization for MDM2 amplification was negative, excluding a dedifferentiated liposarcoma. A diagnosis of stage IIIB leiomyosarcoma of the rectus was made. No adjuvant therapy was administered.

Four months later, computed tomography imaging revealed a new 4.5 cm internal mammary nodal mass, local recurrence of tumor in the remnant left rectus abdominis muscle measuring 4.4 cm, and a large 8.8 cm intra-abdominal mass arising from the omentum, consistent with locally recurrent and metastatic disease. The disease continued to progress despite three cycles of 4-weekly pegylated liposomal doxorubicin, followed by two cycles of 3-weekly gemcitabine and docetaxel.

Tumor tissue from the excision specimen was then sent for molecular profiling using the FoundationOne CDx assay. A MSH6 R922* truncating mutation was found, along with somatic mutations in *TP53*, *BCL2*, and *MLL2* and other truncating mutations in *RB1* and *MSH3*. TMB was 11.35 mutations/Mb, and microsatellite instability (MSI) status was reported as MSI-H.

MSI-PCR performed in our institution showed a shift in three (NR27, BAT25, and BAT 26) of the five MSI marker profiles (Fig 2), confirming the MSI-H status. IHC for DNA mismatch repair proteins (MMR IHC) also showed absent expression for MSH6 protein, whereas MLH1, MSH2, and PMS2 expression was retained (Fig 3). The patient, however, does not have any family history or history of cancer to suggest the possibility of Lynch syndrome.

On review of the excised tumor, it was observed that the tumor has a mild to moderate infiltrate of lymphocytes and plasma cells, especially at the

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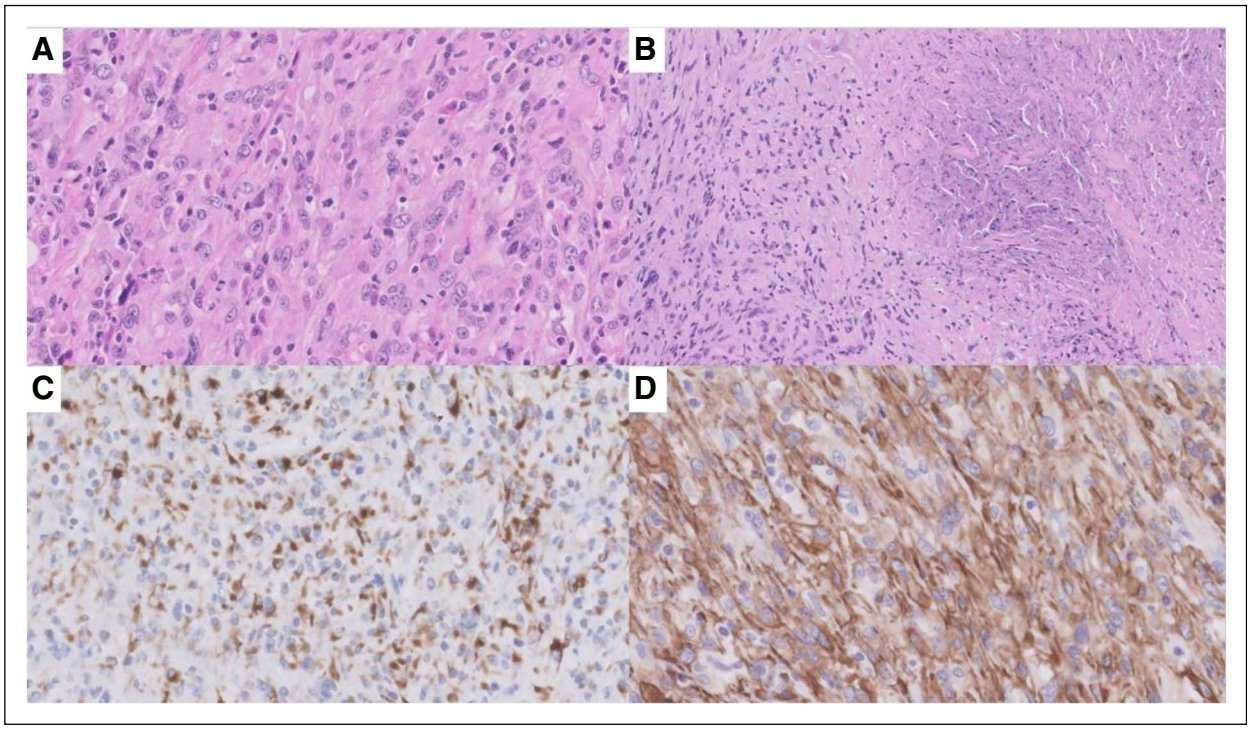

FIG 1. Histology of the excised abdominal wall tumor shows fascicles of atypical spindle cells with (A) eosinophilic cytoplasm and (B) areas of necrosis. Immunohistochemistry shows the tumor cells staining positive for (C) desmin and (D) caldesmon. The features are in keeping with a leiomyosarcoma.

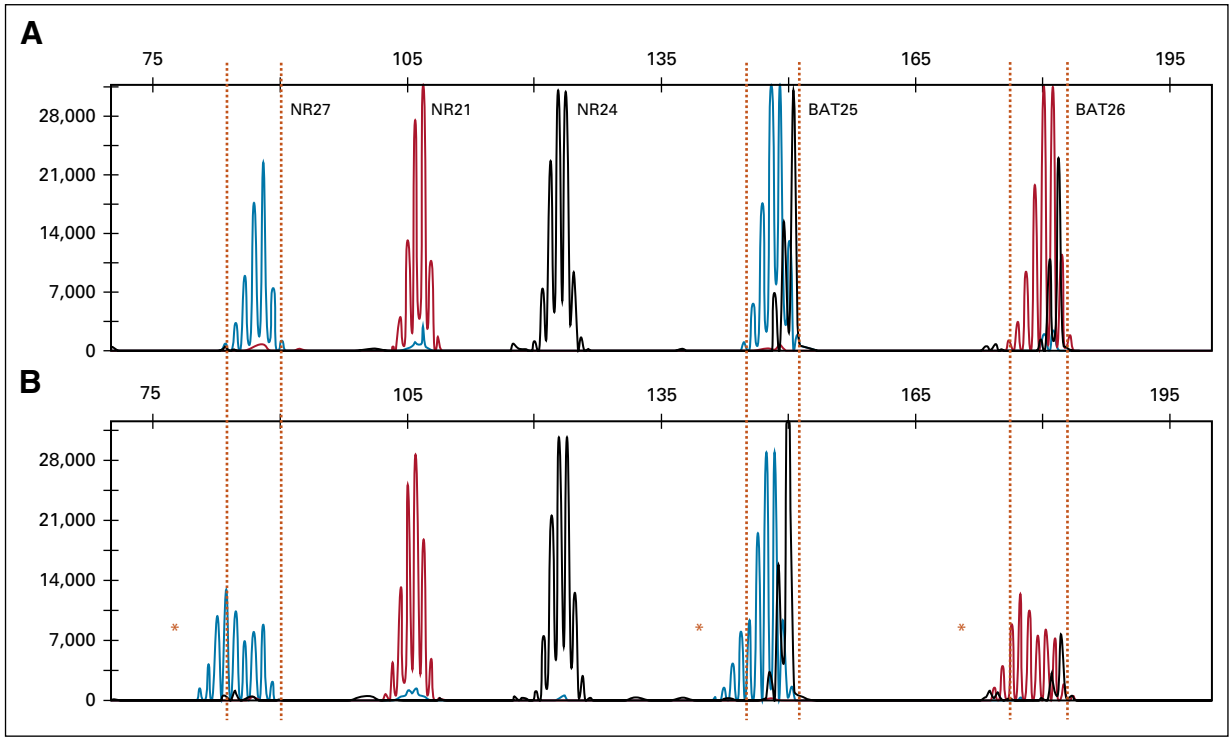

FIG 2. MSI-polymerase chain reaction shows a shift in three of the five MSI marker profiles (NR27, BAT25, and BAT 26) when comparing tumor with normal DNA, in keeping with MSI high status: (A) germline and (B) tumor. MSI, microsatellite instability.

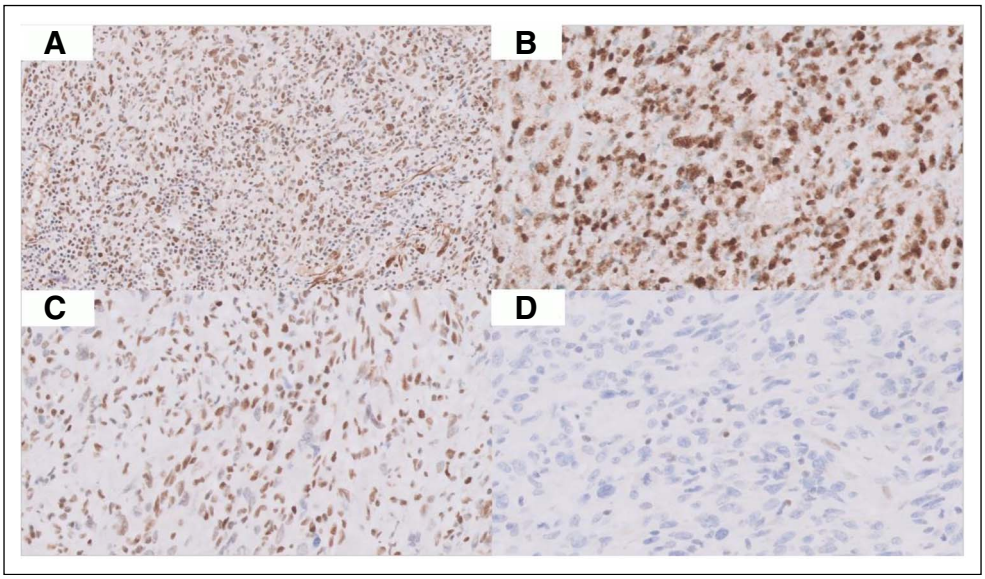

FIG 3. Immunohistochemistry for DNA mismatch repair proteins shows retained expression for (A) MLH1, (B) PMS2, and (C) MSH2, whereas (D) MSH6 was lost.

tumor-stroma interface, in keeping with TILs. There was, however, no aggregation of the lymphoid cells to suggest the presence of tertiary lymphoid structures. IHC for CD3 and CD20 showed that a majority of TILs are CD3+ T cells with a smaller number of CD20+ B-cells (Figs 4A-4C).

PD-L1 (22C3 clone) IHC showed staining of both the tumor cells and the immune cells with an estimated Tumor Proportion Score of 40% and a Combined Positive Score of 50 (Fig 4D).

Direct visualization of the tumor-infiltrating immune cells with multiplex IHC/immunofluorescence showed that the immune cells include CD8+ T cells (CD8-positive, 15.6%) and macrophages (CD68-positive, 18.5%). Regulatory T cells (FOXP3-positive, 3.3%) were relatively infrequent.

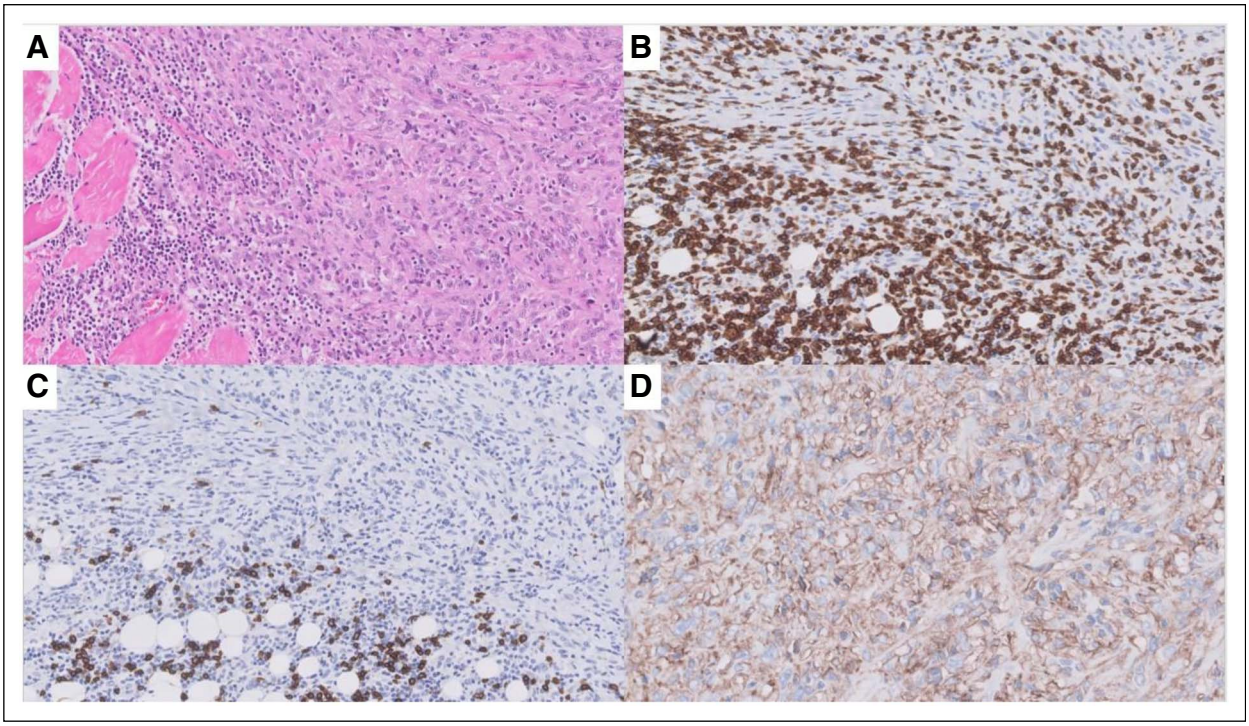

FIG 4. (A) The tumor has a mild to moderate infiltrate of lymphocytes and plasma cells, especially at the tumor-stroma interface, in keeping with TILs. IHC for CD3 and CD20 showed that (B) most of the TILs are CD3+ T-lymphocytes, whereas (C) CD20+ B-lymphocytes are present in smaller numbers. Programmed death ligand-1 (22C3 clone) IHC showed (D) membranous staining of the tumor cells. The estimated Tumor Proportion Score of the tumor was 40%, whereas the Combined Positive Score was 50. IHC, immunohistochemistry; TIL, tumor infiltrating lymphocyte.

PD-L1–positive cells (19.7%) were diffusely scattered throughout the tumor (Fig 5).

The patient then received palliative radiation therapy to the internal mammary nodal mass, before commencing on pembrolizumab 100 mg administered every 3 weeks following the knowledge that the tumor was MSI-H. This dosage of pembrolizumab was chosen because of the cost of the drug, and prevailing local reimbursement policies made it more affordable for the patient at this dosage. After three cycles, imaging showed a decrease in size of both the internal mammary adenopathy (6.3 cm to 4.4 cm) and omental mass (12.1 cm to 11.2 cm), the latter now contiguous with the rectus tumor and containing specks of gas suggestive of fistulation into adjacent bowel (Figs 6A and 6B). The patient also developed progressive subcutaneous abdominal wall abscess and small bowel intestinal obstruction. A decision was then made for the patient to undergo wide excision of the omental/rectus tumor with en bloc small bowel resection and subtotal colectomy.

Histologic examination of the omental/rectus tumor, however, showed extensive necrosis with no residual viable tumor, in keeping with pathologic complete response (Figs 6C and 6D).

Postsurgery, the patient underwent 10 cycles of pembrolizumab. A surveillance scan at 3 months and 6 months postsurgery did not reveal any local or distant disease recurrence, whereas the internal mammary nodal mass remained stable in size.

Discussion

The incidence of MSI-H and MMRd in sarcomas reported in the published literature varies widely from $< 1\%$ to $> 25\%$.[5-15] The contradictory findings from the different studies are likely due to many factors including difference in sample sizes, heterogeneity of the sarcoma subtypes studied, and difference in methodology used including the type of microsatellite marker. The studies that report a higher incidence of MSI-H/MMRd status tend to be older studies with smaller sample sizes, whereas more recent contemporary studies or studies with larger sample sizes tend to report an incidence of around 1%-2%, which is likely more reflective of the true incidence.[7,13-15] Most MSI-H/MMRd sarcomas contain mutations in *MSH2* or *MLH1*, whereas *MSH6* mutations (which was seen in our case) and *PMS2* mutations form the minority, with an incidence of 11.6% and 2.3% of cases, respectively, in one study.[16]

Data on how MSI-H/MMRd status correlates with response to immune checkpoint inhibitor (ICI) therapy in sarcomas remain scant. In a recent study by Doyle et al,[14] three of seven MMRd sarcomas were treated with pembrolizumab. One patient had stable disease, whereas another had disease progression after 4 months of treatment. The third patient died from rapidly progressive disease postsurgical resection after receiving pembrolizumab for only 1.5 months. In another case report by Wang et al,[17] a patient with a MSI-H uterine leiomyosarcoma showed partial response to pembrolizumab after three cycles but ultimately had disease progression after seven cycles. Our case therefore adds to the existing published data of response of MSI-H/MMRd sarcoma to ICI, albeit showing an extremely favorable response. Other case reports of sarcomas showing complete or partial response to ICI do not report the MSI-H/MMRd status.[18-21]

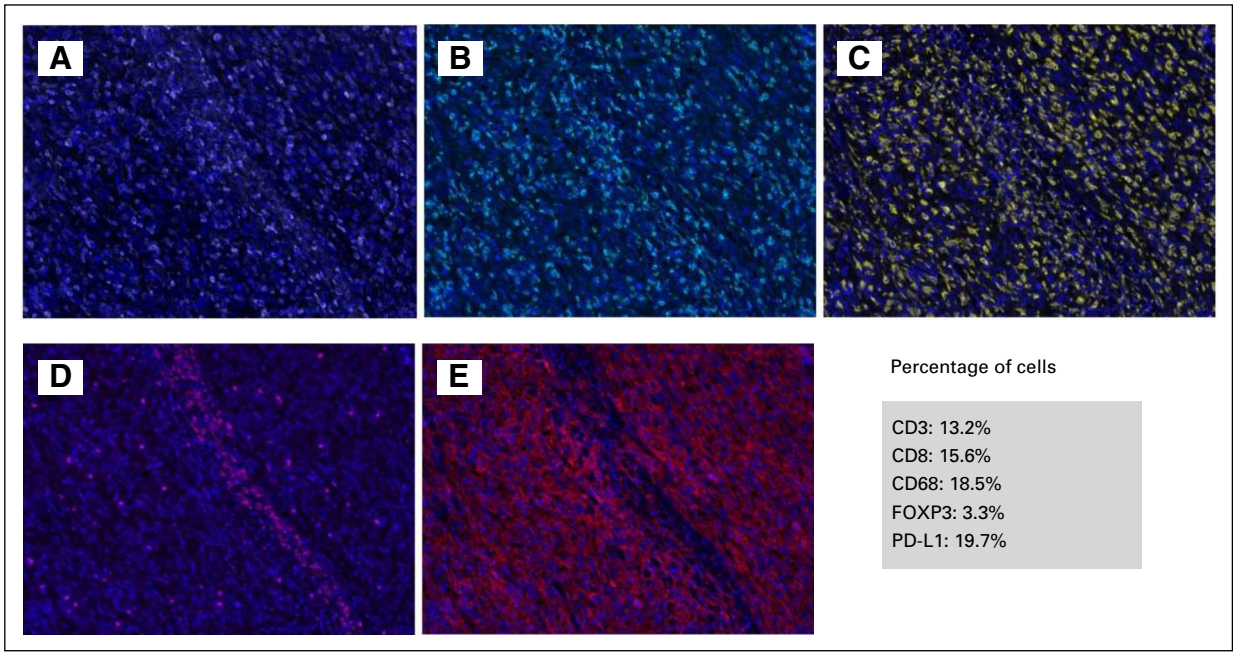

FIG 5. Multiplex immunohistochemistry/immunofluorescence applied to a section of the tumor showed various tumor-infiltrating immune cells: (A) CD3, (B) CD8, (C) CD68, (D) FOXP3, and (E) PD-L1. PD-L1–positive cells (19.7%) were also present. PD-L1, programmed death ligand-1.

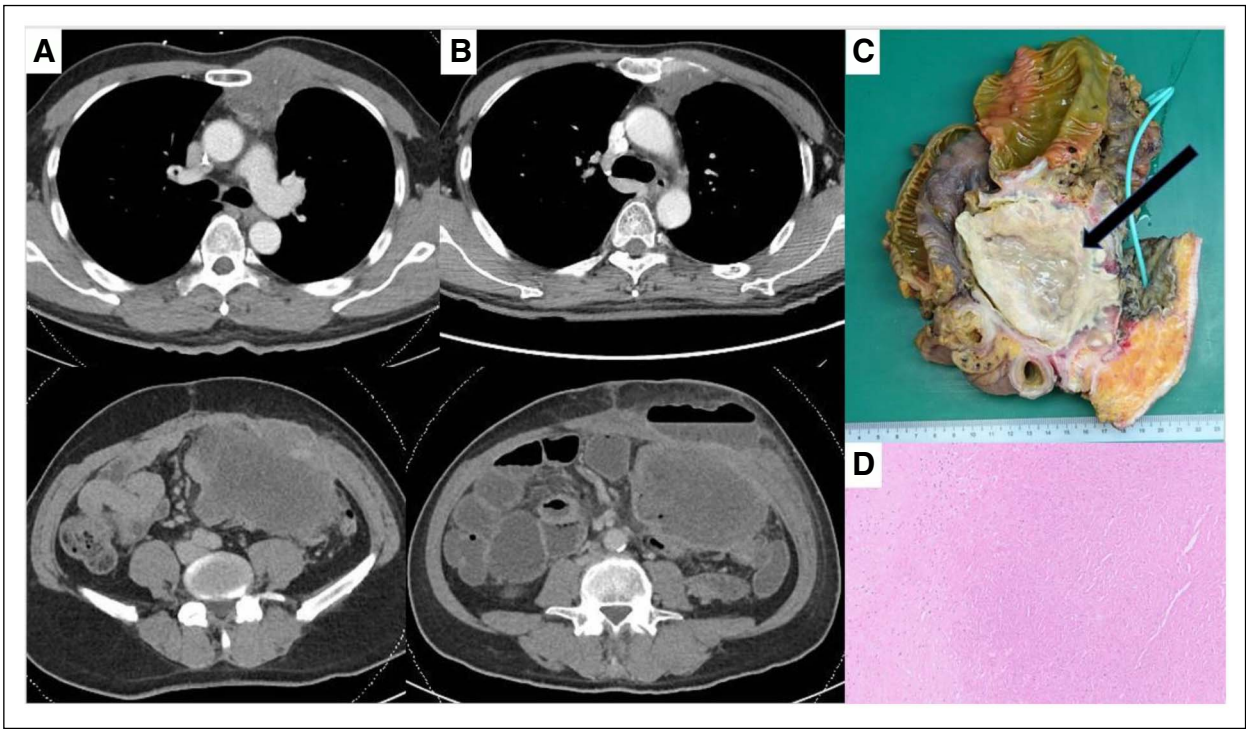

FIG 6. Computed tomography scans (A) before and (B) after three cycles of pembrolizumab treatment, showing decrease in size of the internal mammary adenopathy and marginal decrease in size of the omental metastasis, which is now contiguous with the recurrent rectus tumor. Excision of the omental/rectus tumor, however, showed (C) an extensive necrotic tumor (indicated with arrow), which was confirmed microscopically to harbor (D) no residual viable tumor, in keeping with pathologic complete response.

Data correlating the presence of MSI-H/MMRd status with TMB are also lacking in sarcomas. In the study by Doyle et al,[14] six of the seven MSI-H/MMRd sarcomas had a TMB of $>$ 10 mutations/Mb (ranging from 12.2 to 25.9) with the remaining tumor having a TMB of 9.1. The TMB in MMRd sarcomas was observed to be significantly higher than that in MMR-proficient sarcomas (median mutational burden of 16 *v* 4.6, $P < .001$). This suggests that MSI-H/MMRd sarcomas are likely to have a TMB that exceeds 10. The correlation of MSI-H/MMRd status with PD-L1 expression in sarcomas is more variable. Of the seven MSI-H/MMRd sarcomas in the same study, three had 0%-2% of intratumoral immune cells showing PD-L1 expression, whereas the remaining four had PD-L1 expression in 10%-50% of the intratumoral immune cells. The three tumors with low PD-L1 expression were the three tumors that were treated with pembrolizumab in the study, which may explain their nonresponse to the drug despite the MSI-H/MMRd and high TMB status of the tumors (two of the three cases had TMB of 13.7 and 16.0, whereas the remaining had TMB of 9.1). However, response to ICIs has been reported to occur even in the absence of PD-L1 expression.[22-25] In addition, an angiosarcoma with a low TMB showing complete response to an ICI has also been reported, suggesting that TMB is also not entirely reliable in predicting response to immunotherapy.[26] In a study by Lam et al,[15] the authors found that the level of PD-L1 expression also correlated with the degree of T-lymphocyte infiltration in the tumor, an observation echoed by other studies.[27-30] In the SARC028 phase II clinical trial, patients with advanced sarcomas that responded to pembrolizumab had higher densities of activated T cells ($CD8^+CD3^+PD\text{-}1^+$) and increased percentage of tumor-associated macrophages expressing PD-L1 compared with nonresponders.[31]

Our case of leiomyosarcoma has a relatively high expression of PD-L1 in the tumor cells in addition to MSI-H/MMRd status and elevated TMB of $>$ 10 mutations/Mb. Although MSI-H/MMRd status is not a guarantee of response to ICI as seen in the study by Doyle et al, it is uncertain if the marked response to ICI in our case is due to the high PD-L1 expression or high TMB. Each biomarker on its own has been found to be not always predictive of ICI response as discussed above, and it is likely that the constellation of factors of MSI-H/MMRd status, high TMB, high expression of PD-L1 in the tumor cells, and even the presence of TILs has poised the immune cells to greatly destroy the tumor in this case when immune checkpoint blockade was applied. This suggests that perhaps a multifactorial assessment of MSI-H/MMRd status, TMB, PD-L1 expression, and TILs may be more useful in predicting ICI response in sarcoma than relying on one parameter alone. More study will be required to further refine the use of biomarkers in predicting ICI response in sarcomas.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Administrative support: Jason Yongsheng Chan
Provision of study materials or patients: Timothy Kwang Yong Tay, Xin Xiu Sam, Johnathan Xiande Lim, Jason Yongsheng Chan
Collection and assembly of data: All authors
Data analysis and interpretation: Timothy Kwang Yong Tay, Joe Poh Sheng Yeong, Eileen Xueqin Chen, Jason Yongsheng Chan
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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