

Manipulating the Immune System With Checkpoint Inhibitors for Patients With Metastatic Sarcoma

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OVERVIEW

Sarcomas are a rare group of malignant tumors of mesenchymal origin that comprise 1% of all adult cancers. Despite initial surgery, distant metastatic disease will develop in approximately 25% of patients, and standard chemotherapy has limited durable efficacy. There is a dire need for more effective and less toxic therapies for the treatment of metastatic sarcoma. The immune system plays a major role in cancer control and progression. There have been tremendous breakthroughs in other malignancies by manipulating the immune system with checkpoint inhibitors. These agents, either alone or in combination with other approaches such as radiation, chemotherapy, targeted agents, or immunotherapeutics, have generally led to improved efficacy in selected malignancies thus far. Although promising, these drugs can cause specific immune-related adverse events that require prompt recognition and treatment. In addition, characterizing response and progression radiographically has become somewhat more challenging. Identifying predictive biomarkers of benefit will be essential. There remains optimism and hope that the strides made in other cancers will be emulated in sarcoma.

Sarcomas are a rare group of malignant tumors of mesenchymal origin that comprise 1% of all adult cancers. There are over 50 histologic subtypes. Most patients undergo curative surgical resection with or without adjuvant radiation and chemotherapy.¹ Adjuvant chemotherapy is generally reserved for specific histologic subtypes.² Despite initial surgery, distant metastatic disease will develop in approximately 25% of patients.^{3,4} Complete responses to chemotherapy for metastatic sarcoma are infrequent and the median survival remains dismal.^{5,6} There is a dire need for the development of more effective, less toxic therapies for the treatment of metastatic sarcoma. It has become more apparent that the immune system plays a major role in cancer control and progression. There have been tremendous breakthroughs in other malignancies by manipulating the immune system with checkpoint inhibitors. Therefore, there remains optimism for the development of checkpoint inhibitors in sarcoma.

Immune checkpoints are necessary to prevent autoimmunity and protect tissue from damage when the immune system responds to foreign pathogens.⁷ Alternatively, tumors often overexpress immune checkpoint proteins as a mechanism to avoid death.⁷ As a result, dysregulation of these immune checkpoint pathways lead to tumor immune resistance. Immune checkpoints are typically ligand-receptor interactions, which are amenable to blockade by antibodies.

CTLA-4 BLOCKADE

The CTLA-4 receptor was one of the first receptors to be targeted.⁸⁻¹⁰ CTLA-4 binds to CD28 to downregulate T-cell function (Fig. 1).^{9,11-14} There are two human monoclonal antibodies, ipilimumab and tremelimumab, that have been used to target CTLA-4. Ultimately, ipilimumab was approved by the U.S. Food and Drug Administration (FDA) for metastatic melanoma, and recent data have demonstrated durable long-term survival with 20% of patients surviving beyond 3 years.¹⁵

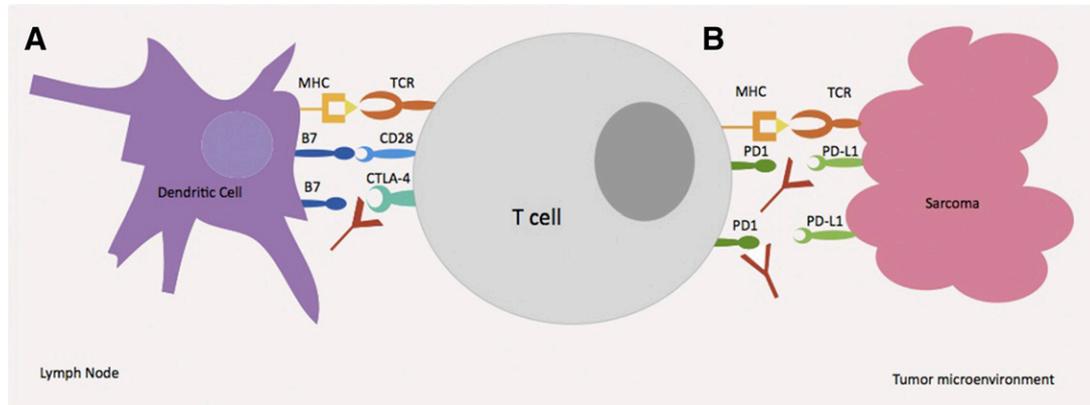
Limited clinical data are available regarding the efficacy of CTLA-4 blockade in sarcoma. In a small phase II study, six patients with synovial sarcoma received 3 mg/kg of ipilimumab every 3 weeks.¹⁶ Four patients completed three doses of ipilimumab, whereas two patients each received one and two doses because of clinical or radiologic progression. There were no documented responses. The patients had rapidly progressive disease and their median survival was only 8.75 months, which is much worse than the average patient with metastatic synovial sarcoma. Given their advanced state, there may not have been sufficient time to see a potential benefit from the ipilimumab, which can typically take time. Further, radiologic progression after just one dose of ipilimumab is not necessarily consistent with true progression in the setting of immunotherapy, and lack of immediate response may not translate to lack of benefit.

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FIGURE 1. Mechanism of Action of CTLA-4 and PDL1 Blockade

(A) Interactions between the tumor antigen on the dendritic cell with the TCR, along with interaction of the costimulatory molecules B7 and CD28, are necessary for T-cell activation. As a negative regulator of the immune response, CTLA-4 competes with CD28 to bind with B7. Ipilimumab and tremelimumab are monoclonal antibodies that bind to CTLA-4 and inhibit negative regulation and activate T cells. (B) The role of the PD-1 receptor is generally more important in the tumor microenvironment during T-cell activation. Antigen exposure leads to expression of PD-1 on the T cells. Interaction of the PD-1 receptor with its ligands PD-L1 and PD-L2 results in negative regulation of T cells in the tumor microenvironment. Antibodies that bind to PD-1 or PD-L1 lead to activation of T cells.

Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor.

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PD-1 BLOCKADE

PD-1 and its ligand PD-L1 is another immunologic checkpoint pathway that serves as a negative regulator of the immune response. There are two ligands, PD-L1 and PD-L2, that are specific for PD-1 and, upon binding, downregulate T-cell activation (Fig. 1).^{17,18} There are multiple antibodies that target PD-1 (e.g., nivolumab, pembrolizumab, and pidilizumab) or PD-L1 (e.g., atezolizumab, durvalumab, and avelumab). Thus far, nivolumab was FDA approved for metastatic melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma. Pembrolizumab was FDA approved for metastatic melanoma that progressed during treatment with ipilimumab or a BRAF-inhibitor, as well as for NSCLC that is PD-L1-positive as confirmed by the PD-L1 immunohistochemistry (IHC) 22C3 pharmDx test.^{19,20} Since then, pembrolizumab is now approved in the front-line setting for

patients with metastatic melanoma. Atezolizumab, durvalumab, and avelumab have been studied in multiple malignancies including bladder cancer, head and neck cancer, gastrointestinal (GI) malignancies, and Merkel cell carcinoma.²¹⁻²⁴ Most recently, the SARC028 phase II study of the anti-PD-1 antibody pembrolizumab for patients with advanced sarcomas was completed (NCT02301039).

COMBINATION APPROACHES

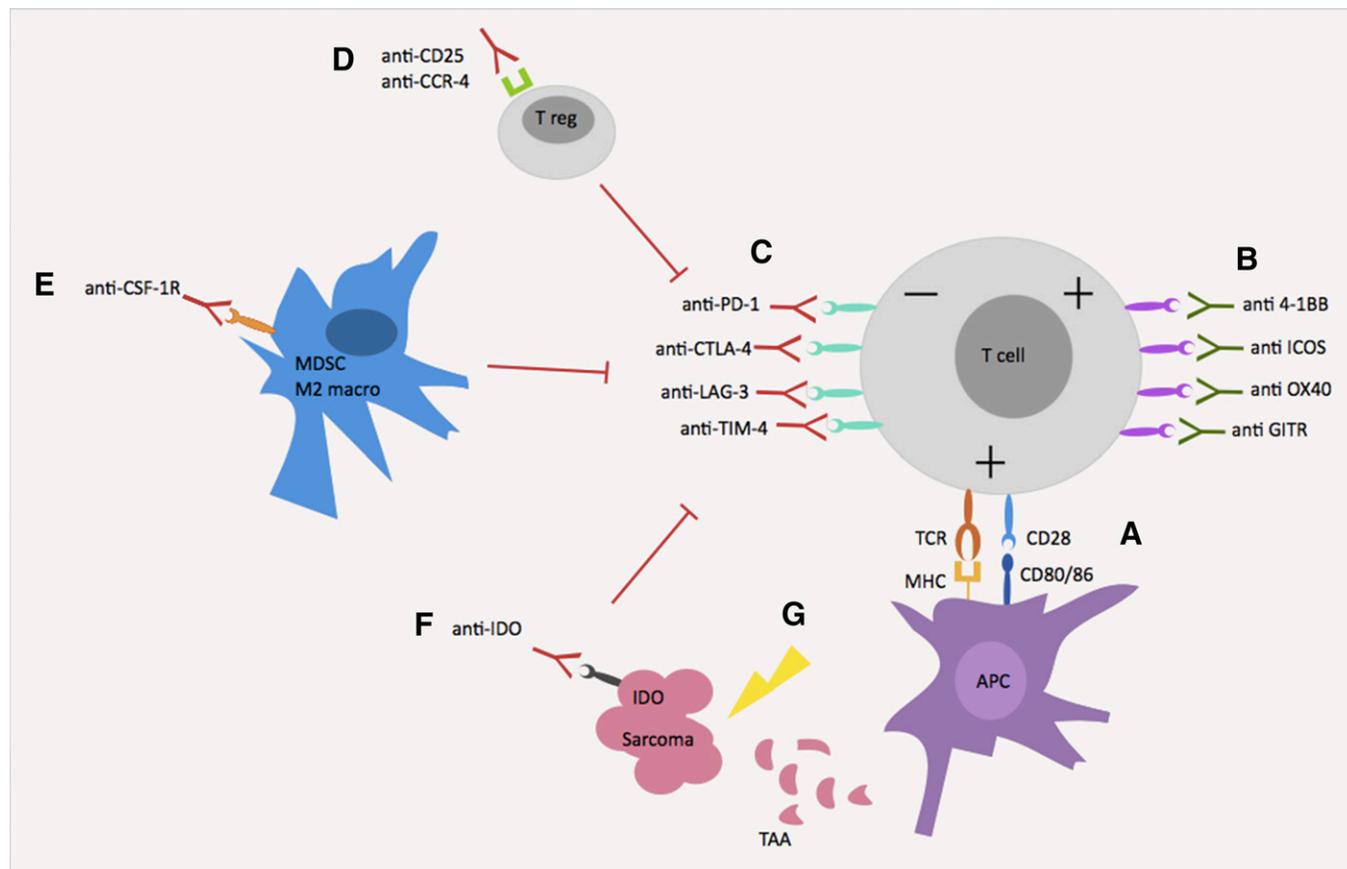
To improve clinical efficacy, there has been much interest in combining checkpoint blockade antibodies with systemic approaches such as chemotherapy, targeted therapy, radiation therapy, and other immunotherapeutics (Fig. 2).

Chemotherapy can alter the immune milieu by reducing myeloid-derived suppressor cells,²⁵ increasing the ratio of M1 to M2 macrophages, and increasing CD8+ T cells and natural killer (NK) cells.^{26,27} Trabectedin has been shown to synergize with an anti-PD-1 antibody in a mouse ovarian cancer model.²⁸ As trabectedin is now FDA approved for selected sarcomas, there may be rationale to explore this combination. There is an ongoing study of pembrolizumab with chemotherapy (pembroplus; NCT02331251). Regardless, it is essential to be mindful of the increased risk of adverse events. For example, in melanoma and NSCLC, some studies that combined chemotherapy with ipilimumab demonstrated an increased risk of transaminitis.²⁹

Targeted therapies such as tyrosine kinase inhibitors (TKIs) can stimulate immune cells, including CD4+ and CD8+ T cells,^{30,31} NK cells,³² and dendritic cells.³³ Combined blockade of CTLA-4 plus KIT via imatinib or dasatinib led to an improved antitumor effect in a murine model of GI stromal tumor (GIST).³⁴ This provided the scientific rationale to launch a phase I study of dasatinib plus ipilimumab in patients with advanced GIST and other sarcomas.³⁵ The maximum tolerated dose was identified as 70 mg of

KEY POINTS

- The CTLA-4 receptor was one of the first checkpoint inhibitors to be targeted with limited exploration and efficacy in sarcoma.
- The PD-1/PD-L1 immunologic checkpoint pathway has demonstrated efficacy in many malignancies; studies are ongoing in sarcoma.
- There is substantial interest in combining checkpoint blockade antibodies with multiple approaches including chemotherapy, targeted therapy, radiation therapy, and other immunotherapeutics.
- Understanding, recognizing, and promptly treating immune-related adverse events is essential as these immunotherapeutics continue clinical development.
- Identifying biomarkers predictive of benefit is essential to continue to move the field forward.

FIGURE 2. Strategies to Enhance Antitumor Immunity

Immunotherapy with checkpoint inhibitors for sarcoma can be approached in many different ways. Various aspects of the immune response can be targeted. (A) T-cell activation requires interaction of TCR with MHC and interaction of costimulatory molecules, such as CD28 and CD80/86. (B) Enhanced T-cell activation can occur with costimulatory agonists such as antibodies that target 4-1BB, ICOS, OX40, or GITR. There are many pathways that can lead to downregulation of the immune, as noted on the left side of the figure. (C) Inhibition of the immune response can be mitigated by blocking checkpoint inhibitors beyond PD-1 and CTLA-4, such as LAG-3 or TIM-4, (D) or by decreasing regulatory T-cells with antibodies that target CD25 or CCR-4. (E) Chemotherapy can alter the ratio of M1 to M2 macrophages and potentially deplete MDSC. Antibodies that bind colony stimulating factor-1R may also deplete M2 macrophages. (F) IDO is a negative regulator of the immune response found in the tumor microenvironment that can be targeted with anti-IDO antibodies. (G) Increased exposure of tumor-associated antigens with radiation, as well as chemotherapy, can ultimately enhance the immune response.

Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; APC, antigen-presenting cell; ICOS, inducible T-cell costimulator; GITR, glucocorticoid-induced tumor necrosis factor receptor–related protein; TIM-3, T-cell immunoglobulin and mucin domain–containing; LAG-3, lymphocyte-activation gene; T reg, regulatory T cell; CCR4, chemokine C-C Motif receptor 4; MDSC, myeloid derived suppressor cells; CSF-1R, colony stimulating factor 1 receptor; IDO, indoleamine 2,3-dioxygenase; TAA, tumor-associated antigen.

Adapted from Zamarin D, Postow MA. Immune checkpoint modulation: rational design of combination strategies. *Pharmacol Ther.* 2015;150:23-32.

dasatinib per day administered concurrently with 10 mg/kg of ipilimumab. In this treated cohort, three of eight patients with heavily treated GIST and one of five patients with soft tissue sarcoma achieved durable disease control. There is an ongoing expansion trial using the maximum tolerated dose (NCT01643278). In renal cell carcinoma, nivolumab with sunitinib or pazopanib was found to be more efficacious, albeit with increased hepatic and renal toxicity.³⁶ Therefore, TKIs must be cautiously combined with PD-1 inhibitors. There is an ongoing clinical trial of pembrolizumab and axitinib for selected sarcomas (NCT02636725).

Radiotherapy has been shown to increase antigenic expression and increase specific immune responses. In reported case series of patients with melanoma, the so-called abscopal effect has resulted in decreased metastatic disease sites after local radiation.³⁷ There are ongoing clinical trials investigating the safety and efficacy of checkpoint blockade with radiation therapy.

Most recently, combined CTLA-4 and PD-1 blockade with ipilimumab and nivolumab was FDA approved for metastatic melanoma. In the trial that led to its approval, the combination approach resulted in an overall response rate of 61% compared with 11% in the ipilimumab alone group.³⁸ These exciting findings have led to an interest in exploring these agents in multiple disease types. The phase II study of nivolumab with or without ipilimumab for metastatic sarcoma has nearly completed accrual and data analysis is pending at this time (NCT02500797). Beyond combining PD-1 with CTLA-4 blockade, there are ongoing efforts of fine-tuning the immune response with various costimulatory molecules (OX-40, glucocorticoid-induced tumor necrosis factor receptor–related protein [GITR], CD28, 4-1BB, CD27) or co-inhibitory molecules (T cell immunoglobulin, mucin domain–containing [TIM-3], and lymphocyte-activation gene [LAG-3]).³⁹⁻⁴² There are several immune inhibitory mechanisms that can be targeted as well, including

tumor-infiltrating regulatory T cells via inhibition of chemokine receptor 4 (CCR-4) or CD25, tumor-associated macrophages, and myeloid-derived suppressor cells via anticolonystimulating factor-1 receptor (CSF-1R).^{43,44} Indoleamine 2,3-dioxygenase (IDO) is expressed on tumor cells and is upregulated in many malignancies. IDO is an essential enzyme that depletes metabolites necessary for immune function. There are inhibitors of IDO being evaluated in clinical trials.^{45,46} There is rationale for targeting these mechanisms in combination with PD-1/PD-L1 blockade, and studies are currently underway to evaluate these strategies in different tumor types. As these clinical trials begin to unfold, being cognizant of the potential additive immune-mediated adverse events will be critical.

UNDERSTANDING AND MONITORING IMMUNE-RELATED SIDE EFFECTS

Unique patterns of adverse events have been observed in patients treated with checkpoint blockade inhibitors. As a result of nonspecific immune activation, these drugs can result in various adverse events referred to as immune-related adverse events. These events can affect multiple organ systems, including, but not limited to, dermatologic, pulmonary, hepatic, renal, neurologic, endocrine, and GI.^{47,48} Grade 3 or 4 events have been reported in 10% to 20% of patients treated with CTLA-4 or PD-1/PD-L1 blockade.^{47,48} It seems as though the incidence of adverse events may be somewhat lower with anti-PD-1/PD-L1 antibodies because their effects tend to occur peripheral to the tumor, whereas CTLA-4 blockade effects tend to occur more centrally. Generally, the approach in treating these adverse events is early recognition. Depending on severity, withholding treatment and immediate initiation of immunosuppression, typically with corticosteroids, is essential. For patients whose disease is refractory to corticosteroids treatment, tumor necrosis factor α antagonists (infliximab) is commonly used for diarrhea/colitis or mycophenolate mofetil for transaminitis. Following established algorithms is essential to ensure these adverse events are addressed appropriately.^{47,49}

MEASURING CLINICAL RESPONSE: IMMUNE-RELATED RESPONSE CRITERIA

When patients pursue cytotoxic chemotherapy, RECIST can be used to effectively determine changes in tumor burden.⁵⁰ When treated with immunotherapy agents, patients can sometimes develop an increase in tumor burden or new lesions followed by subsequent decrease or disease stabilization. Per RECIST or World Health Organization (WHO) criteria, this would qualify as disease progression. Interestingly, of 123 patients with metastatic melanoma treated with ipilimumab who progressed at week 12, almost 20% went on to have a partial response or stable disease.⁵¹ In another analysis of patients who were long-term survivors of melanoma who had received ipilimumab, nearly 25% had progressive disease per WHO criteria, yet had survived 4 years after completion of therapy.⁵² This prompted an interest in re-evaluating response criteria for patients treated with immunotherapy. The immune-related

response criteria (irRC) define progressive disease as total disease growth up to 25% from baseline or total disease burden (new lesions plus the target lesion) greater than 25%.⁵¹ These criteria have correlated with overall survival for patients with melanoma who were treated with anti-CTLA-4 blockade.⁵¹ Much of the experience with irRC has been developed specifically in patients who were treated with CTLA-4 therapy (Table 1). One would anticipate similar findings with PD-1/PD-L1 blockade. However, generally the median time to response tends to be more rapid and higher with PD-1/PD-L1 blockade. Nonetheless, these criteria must be prospectively validated to ultimately serve as a surrogate for efficacy with these agents.

BIOMARKERS ASSOCIATED WITH OUTCOME

It is clear that predictive biomarkers of benefit with checkpoint blockade are required for ultimate optimal patient selection. There was initial suggestion that PD-L1 expression may correlate with clinical activity of PD-1 blockade.⁵³ The definition of positive PD-L1 expression by IHC has varied based on the assay and disease type. Expression of PD-L1 in sarcoma has been performed with at least three different antibodies, and the results have varied. One series, using the rabbit monoclonal antihuman PD-L1 antibody (clone 28-8) and an automated assay developed by Dako identified greater than 1% PD-L1 expression in six out of 50 (12%) samples.⁵⁴ Another group used the CD274/B7-1H antibody to determine PD-L1 expression by IHC in 161 samples of osteosarcoma, 46 samples of leiomyosarcoma, and 33 samples of Ewing sarcoma.⁵⁵ Positive PD-L1 expression was noted in 36% of osteosarcoma samples, 97% of leiomyosarcomas, and 39% of Ewing sarcomas. Finally, a third group tested PD-L1 expression in 105 cases of soft tissue sarcoma with the Santa Cruz antibody. In their study, 58% of soft tissue sarcoma had intratumoral infiltration of PD-1–positive lymphocytes and 65% of soft tissue sarcoma expressed PD-L1. Lymphocyte and macrophage PD-L1 expression was noted in 30% and 58%, respectively, and lymphocyte and macrophage infiltration was present in 98% and 90%, respectively. There was variability and a wide range of types of lymphocytes present (e.g., CD3, CD4, CD8, FoxP3.) It is challenging to understand the significance of these particular values and what defines high or low in sarcoma. In this study, lymphocyte and macrophage infiltration was noted to be fairly common in sarcoma, while PD-L1 tumor expression is uncommon in sarcoma, with the highest frequency observed in GIST.

Although the initial phase I trial of nivolumab in solid tumors suggested that tumors with PD-L1 expression by IHC may benefit more frequently from PD-1 blockade,⁵³ more recent data from the combination ipilimumab plus nivolumab trial in metastatic melanoma have demonstrated that lack of PD-L1 expression did not preclude benefit.⁵⁶ Since then, there have been data presented in multiple malignancies that have demonstrated benefit of PD-1 blockade, regardless of tumor PD-L1 expression.^{22,23,56,57} The role of PD-L1 expression as a predictive biomarker of benefit with anti-PD-1 therapy continues to evolve. Better biomarkers are necessary to help identify which patients are most likely to benefit.

TABLE 1. Comparison of RECIST 1.1, World Health Organization, and Immune-Related Response Criteria⁵¹

	RECIST 1.1	WHO	irRC
New Measurable Lesions ($\geq 5 \times 5$ mm)	PD	PD	Added into tumor burden
New Nonmeasurable Lesions ($\leq 5 \times 5$ mm)	PD	PD	Does not define PD, but will not allow irCR
Nonindex Lesions	Contribute to BOR	Contribute to BOR	Contribute to irRC
Complete Response	Disappearance of all lesions	Disappearance of all lesions*	Disappearance of all lesions*
Partial Response	At least a 30% decrease in sum of diameters of target lesions, no new lesions, or unequivocal progression in nonindex lesions	A \geq 50% decrease in SPD, no new lesions, or unequivocal progression in nonindex lesions*	A \geq 50% decrease in tumor burden compared with baseline*
Stable Disease	Does not meet criteria for PR or PD, no new lesions or unequivocal progression of nonindex lesions	Does not meet criteria for PR or PD	Does not meet criteria for PR or PD
Progressive Disease	At least a 20% increase in sum of the diameters of target lesions, smallest sum from study is used as reference. Sum must demonstrate an increase of at least 5 mm. One or more new lesions considered PD.	At least 25% increase in SPD compared with nadir and/or unequivocal progression of nonindex lesions and/or new lesions	At least 25% increase in tumor burden compared with nadir*

*Confirmation necessary at least 4 weeks apart.

Abbreviations: WHO, World Health Organization; irRC, immune-related response criteria; PD, progressive disease; BOR, best overall response; SPD, sum of the product of the greatest diameter; PR, partial response.

The impact of mutational burden and novel tumor antigen expression (neoantigens) with response to ipilimumab was evaluated in 25 patients with metastatic melanoma who were treated with ipilimumab.⁵⁸ Of these patients, 11 had evidence of long-term benefit as noted by their median overall survival of 4.4 years (range 2–6.9 years) and median duration of response of 59 weeks (range 42–361 weeks). Alternatively, there were 14 patients who had minimal or no benefit; their median overall survival was 0.9 years (range 0.4–2.7 years) without any evidence of radiographic response. In this analysis, high mutational burden was statistically associated with benefit distinguishing long-term responders from nonresponders. However, there were patients with high mutational burden who did not appear to benefit. Using a bioinformatics pipeline, immunogenic mutations were identified that led to the production of specific peptide sequences, which resulted in candidate neoantigens that ultimately correlated with benefit to ipilimumab. Similar findings were noted in NSCLC whereby higher mutational burden in the tumor was associated with improved response, clinical benefit, and progression-free survival.⁵⁹ It is unknown if mutational burden and/or neoantigen formation will correlate with potential benefit to checkpoint inhibitors in sarcoma. Radiation-associated sarcomas presumably do have substantial DNA damage, but most sarcomas are not

characterized by DNA damage caused by ultraviolet exposure or tobacco use. Nonetheless, although some sarcomas are characterized by simple translocations, many do have a fair amount of genomic instability. Exploring mutational burden and possible neoantigen production remains an area of research interest.

CONCLUSION

The field of sarcoma immunotherapy is in its infancy. New successful therapies, such as the checkpoint inhibitors, have begun to transform the care of many patients with metastatic melanoma, lung, and renal cell carcinoma. The success and efficacy of these therapies have led to better understanding of cancer immunology. Moving forward in the sarcoma field, there are many questions that remain unanswered. Continuing to explore the activity of immunomodulatory agents alone or in combinatorial approaches is essential. Being conscious of the potential of immune-mediated adverse events will minimize risk to patients. It will be necessary to continue to optimize the treatment of patients who experience immune-mediated adverse events. Prospective clinical trials will be necessary to further investigate the role of irRC to determine response and progression. Finally, determining and identifying predictive biomarkers will be essential to continue to move the field forward.

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