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MINI REVIEW

Immune checkpoint inhibitors in sarcomas: in quest of predictive biomarkers

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Sarcomas are a rare group of tumors of mesenchymal origin. Metastatic sarcomas are often difficult to treat and unresponsive to standard radio- and chemotherapy, resulting in a poor survival rate for patients. Novel treatments with immune checkpoint inhibitors have been proven to prolong survival of patients with a variety of cancers, including metastatic melanoma, lung, and renal cell carcinoma. Since immune checkpoint inhibitors could provide a novel treatment option for patients with sarcomas, clinical trials investigating their efficacy in these group of tumors are ongoing. However, the discrimination of patients that are the most likely to respond to these treatments is still an obstacle in the design of clinical trials. In this review, we provide a brief overview of the mechanisms of action of immune checkpoint inhibitors and discuss the proposed biomarkers of therapy response, such as lymphocytic infiltration, intratumoral PD-L1 expression, and mutational load in sarcomas.

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Sarcomas are a heterogeneous group of rare neoplasms, originating from somatic mesenchymal tissues, with more than 50 distinct histologic subtypes. Although accounting for $<1\%$ of all adult solid malignant cancers, sarcomas form more than 20% of all pediatric solid malignant cancers.[1] In spite of chemotherapy and radiotherapy, the median survival for metastatic sarcoma is ~ 12 months.[2] Novel treatment options for these patients are therefore of utmost importance.

One class of such potential new therapeutics is the immune checkpoint inhibitors. In 2010, the treatment of advanced melanoma patients with antibodies that block cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) marked the beginning of the successful employment of this new class of immunotherapeutics.[3] Added to dacarbazine, ipilimumab has doubled the 5-year survival rates in patients with advanced melanoma compared with patients treated with dacarbazine alone.[4] A few years later, antibodies directed against programmed death 1 (PD-1) or PD-ligand 1 (PD-L1) demonstrated clinical efficacy in melanoma patients and in other cancer types.[5–10] Moreover, therapies targeting the PD-1/PD-L1 axis produced less immune-related side effects compared to treatment with anti-CTLA-4 antibodies.[11]

These promising immunotherapies may offer new treatment options for sarcoma patients and clinical trials are being performed to explore their potential. Because of the heterogeneity of sarcomas, researchers and clinicians tried to identify which subtypes would be suitable for immunotherapeutic strategies. Considering the genetic background of sarcomas, it was postulated that high-grade sarcomas with complex genomes would be the best candidates for treatment with immune checkpoint inhibitors.[12]

Recently, biomarkers predictive of response to immune checkpoint inhibitors have been proposed and investigated and could therefore guide selection of sarcoma patients for clinical trials[13,14]. In this review, we describe the mechanisms of action of immune checkpoint inhibitors and discuss and summarize the literature on the presence of biomarkers in sarcomas that may predict treatment response. In light of these, we discuss the potential application of immune checkpoint inhibitors in the treatment of sarcomas.

MODE OF ACTION OF IMMUNE CHECKPOINT INHIBITORS

Anti-CTLA-4 Antibodies

Our knowledge on how the immune system combats cancer cells has increased ever since Burnet[15] and Thomas proposed the concept of cancer immune surveillance in the 1950s. To induce an immune response, professional antigen-presenting cells (APCs), most importantly dendritic cells (DCs), take up tumor-associated antigens and migrate via lymphatic vessels to the regional lymph nodes.[16] In the lymph nodes, the DCs can activate naive T cells by presenting tumor antigens in complex with human leukocyte antigen (HLA) class I and II

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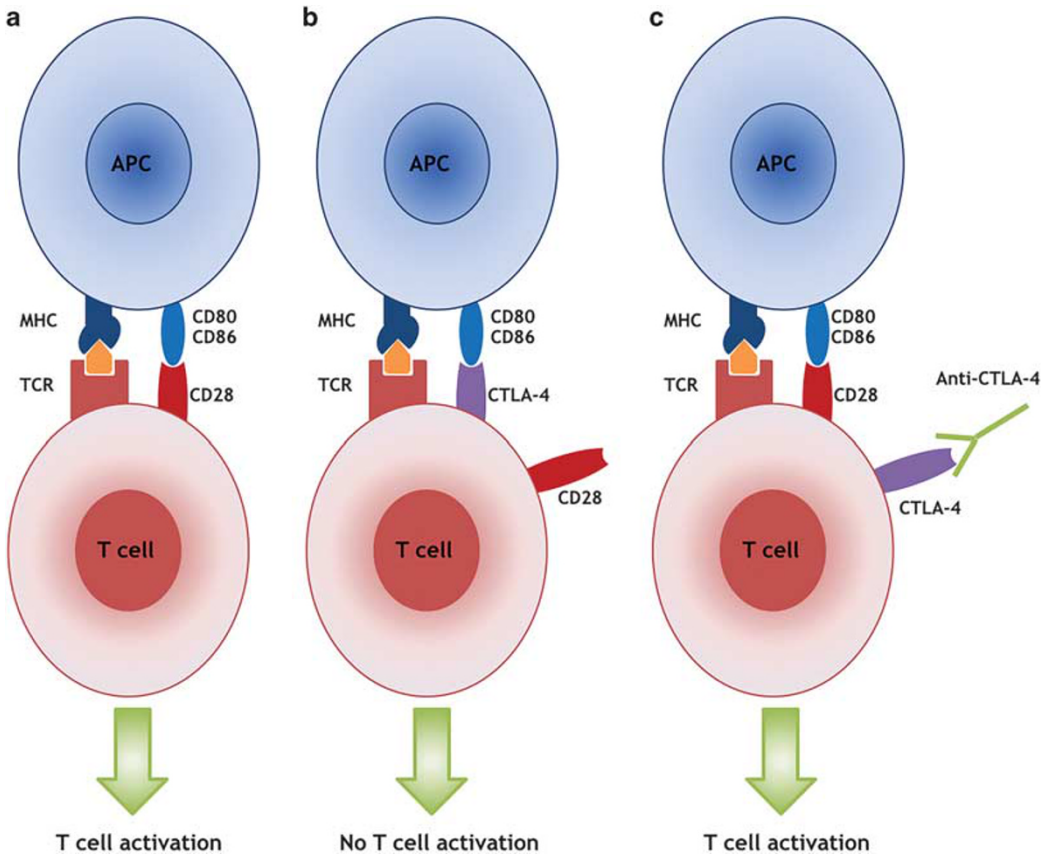

Figure 1 Schematic overview of the mechanism of action of anti-CTLA-4 antibodies. APCs take up tumor antigens in the periphery and migrate to the lymph nodes. In the lymph nodes, the APCs present tumor antigens in complex with HLA class I molecules to naive T cells, which then become primed. Complete activation of the naive T cell occurs when a co-stimulatory signal is provided by binding of CD80 or CD86 on the APC to CD28 on the naive T cell (**a**). However, naive T cells can also express CTLA-4 that, by interacting with CD80 and CD86, transduces an inhibitory signal that leads to T-cell anergy (**b**). Treatment with CTLA-4-blocking antibodies promotes activation of naive T cells, which can then migrate to tumor tissues and directly kill tumor cells ($CD8^+$ T cells) or provide an inflammatory environment (T_H1 $CD4^+$ cells) (**c**).

molecules to the T-cell receptor of naive $CD8^+$ and $CD4^+$ T cells, respectively. This priming of the T cells requires additional co-stimulatory signals by binding of CD28 on the naive T cell to CD80 and CD86 on the APC (Figure 1a).[17] The immune checkpoint molecule CTLA-4, however, can compete with CD28 for binding to CD80 and CD86, and produce inhibitory signals to the activated T cells, acting as a real break and leading to T-cell anergy and apoptosis.[18] Interestingly, regulatory T cells (T_{reg} cells) also need CTLA-4 for their immunosuppressive function, suggesting that anti-CTLA-4 treatment might also interfere with the immunosuppressive function of T_{reg} cells.[19] The importance of CTLA-4 in maintaining a balanced immune response has been demonstrated with CTLA-4 knockout mice that develop severe autoimmune lymphoproliferative disease.[20,21] By releasing such an immunosuppressive brake, treatment with anti-CTLA-4 antibodies result in an enhanced activation of naive T cells. Although APCs can activate both $CD4^+$ and $CD8^+$ T cells, treatment with anti-CTLA-4 antibodies is believed to mainly regulate the activity of $CD4^+$ T cells, which can develop in Th1 $CD4^+$ cells after activation and provide important cytokines, such as IL-2, for the activation of $CD8^+$ T cells. These T cells can then migrate to the tumor tissues and recognize tumor antigens presented in complex with HLA class I at the surface of tumor cells, followed by elimination of the latter.[22]

Anti-PD-1 and Anti-PD-L1 Antibodies

While the anti-CTLA-4 blockade strategy leads to a general enhancement of T-cell priming, antibodies directed against PD-1 and PD-L1 act on T cells that have already been activated, but circulate in the lymph nodes or reside in the tumor microenvironment itself. PD-1 is a surface protein expressed on activated T and B cells while PD-L1 is mostly expressed on APCs, such as macrophages and DCs, and tumor-infiltrating lymphocytes (TILs), but can also be expressed on tumor cells.[23] In normal physiology, the PD-1/PD-L1 axis represents an important immune checkpoint to prevent immune-mediated tissue damage (Figure 2), and PD-1 knockout mice have shown increased susceptibility

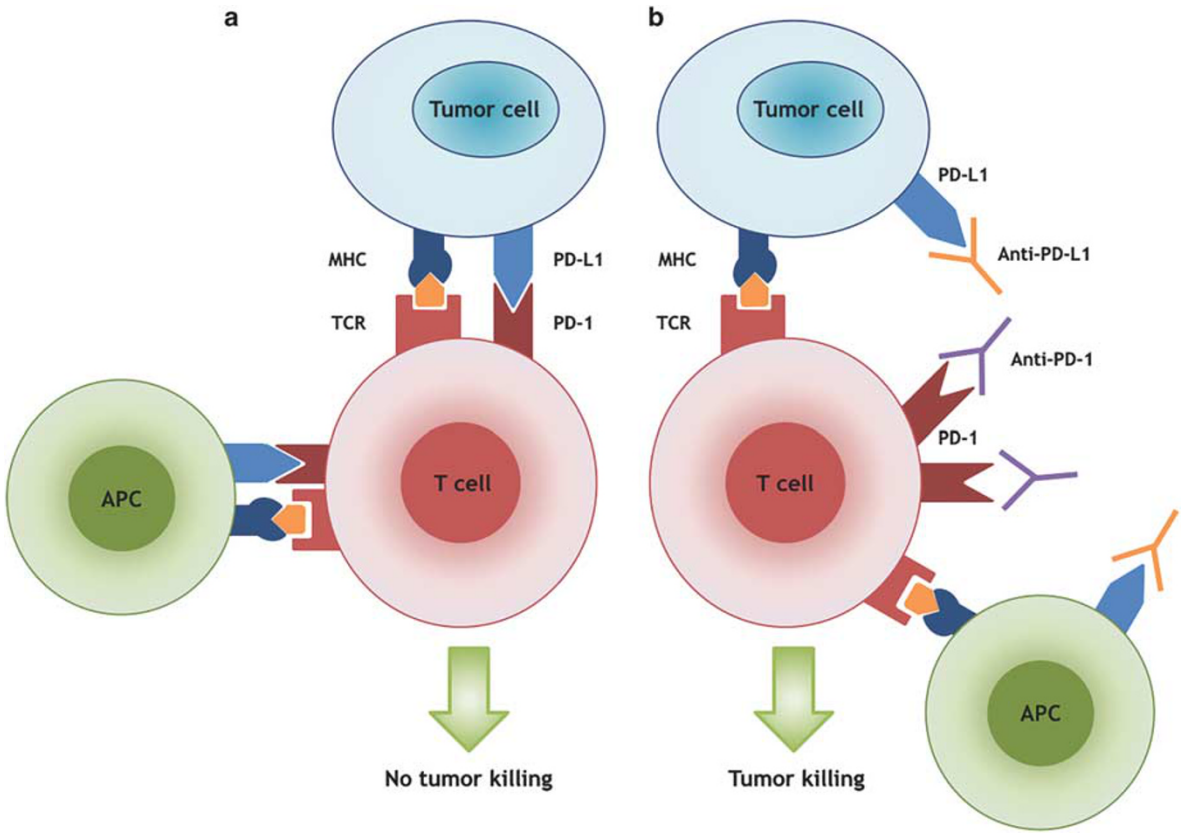

Figure 2 Schematic overview of the mechanism of action of anti-PD-1 and anti-PD-L1 antibodies. Tumor cells can upregulate PD-L1 expression by genetic alterations or chromosomal translocation. INF-γ and other cytokines in the tumor microenvironment can also upregulate the expression of PD-L1 on tumor cells and myeloid cells, such as APCs. PD-L1 on these cells binds to PD-1 on active T cells, inhibiting the T-cell receptor-mediated proliferation of the T cells, leading to reduced killing of the tumor cells. $CD8^+$ T cells can interact with tumor cells and APCs through MHC class I molecules, whereas $CD4^+$ T cells interact with APCs through MHC class II molecules. (**a**) Administration of anti-PD-L1 and anti-PD-1 antibodies prevents immune inhibition by the PD-L1 on the tumor cells or myeloid cells and subsequently enhances tumor killing (**b**).

to the development of autoimmune disorders.[24] As PD-1 is expressed on exhausted T cells, anti-PD-1 therapy can reinforce the immune responses of this subset of T cells.[25] However, PD-1 is also expressed during earlier stages of T-cell activation, where it has an important role in the induction of tolerance against self-antigens and the generation of active cytotoxic T lymphocytes.[26] Of note, PD-1 knockout mice exhibit autoimmunity with a markedly augmented CD8 proliferation.[24] Administration of anti-PD-1 or anti-PD-L1 antibodies aims to release this immunological break and enhance the cytotoxic T-cell response, leading to tumor control or elimination.

Various cancer types like non-small-cell lung carcinoma and melanoma exploit this immunosuppressive interaction and show expression of PD-L1.[23,27,28] For example, the expression of PD-L1 on tumor cells can be the result of genetic alterations.[29] More often, PD-L1 expression on tumor cells, APCs, and other myeloid cells is induced by IFN-γ and other inflammatory cytokines present in the tumor microenvironment.[30] In addition to the PD-1-mediated immunosuppression, many of the tumor-infiltrating myeloid cells, such as myeloid-derived suppressor cells and tumor-associated macrophages (TAMs), show pro-tumorigenic activity. Some sarcomas secrete factors such as colony-stimulating factor-1 (CSF-1) and others to attract and stimulate TAMs, creating an immunosuppressive microenvironment.[31,32] Recent data suggest that TAMs might facilitate tumor resistance against anti-PD-1 and anti-PD-L1 therapy.[33,34] To explore the combination of inhibition of TAMs and enhancement of T-cell-mediated immune responses, an ongoing clinical trial is exploring the combined inhibition of the CSF-1 receptor and PD-1 pathway in GIST and other solid tumors (ClinicalTrials.gov Identifier: NCT02452424).

BIOMARKERS FOR IMMUNOTHERAPY IN SARCOMAS

There is a clear rationale for the use of immune checkpoint inhibitors in sarcomas. Many sarcomas harbor chromosomal translocations resulting in expressed fusion proteins, which can provoke an immunological response. Of note, fusion proteins of synovial sarcoma, clear cell sarcoma, and desmoplasmic small round cell tumor have been

demonstrated to bind HLA class I molecules.[35] In one study, an *in vitro* cytotoxic T-cell response against alveolar rhabdomyosarcoma cells was induced using DCs pulsed with a PAX-FKHR fusion protein breakpoint epitope.[36] Some sarcomas, such as synovial sarcomas and myxoid liposarcomas, also overexpress cancer/testis antigens like NY-ESO-1, which can also trigger $CD8^+$ T-cell-mediated lysis of tumor cells.[37,38] Treatment with anti-CTLA-4, anti-PD-1, or anti-PD-L1 antibodies could enhance $CD8^+$ T-cell-mediated tumor lysis by skipping the crucial immune checkpoints during T-cell priming, activation, and T-cell-mediated eradication of the tumor. Multiple clinical trials are now investigating the potential of immune checkpoint inhibitors as a treatment for sarcoma patients. The first clinical results from the SARC028 study, where patients with advanced sarcomas were treated with the anti-PD-1 antibody pembrolizumab, showed response in patients with undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, synovial sarcoma, osteosarcoma, and dedifferentiated chondrosarcoma.[39] In a cohort of patients with metastatic sarcoma, Paoluzzi *et al*[40] observed partial responses in a dedifferentiated chondrosarcoma, epithelioid sarcoma, and osteosarcoma after treatment with nivolumab. However, in one pilot study including six synovial sarcoma patients, treatment with anti-CTLA-4 antibody did not result in an immunological antitumor response and the disease progressed rapidly in all patients.[41] Therefore, biomarker identification will be fundamental to improve the selection of sarcomas that will respond to therapy with immune checkpoint inhibitors.

Tumor-Infiltrating Lymphocytes

The number and type of TILs could serve as a predictive biomarker for treatment with immune checkpoint inhibitors. In a cohort of patients with metastatic melanomas, patients responding to anti-PD-1 treatment were shown to have a higher number of $CD8^+$ TILs before treatment.[42] This finding clearly indicates that an ongoing antitumor immune response present before treatment is important for the clinical outcome of treatment with anti-PD-1 therapy. Furthermore, in many cancers the presence of TILs often serves as a prognostic factor for patient survival. In patients with colon cancer, higher T-cell infiltration in the tumor was shown to be a predictive biomarker for disease-free survival.[43] A high count of TILs also associated with better overall survival in melanoma patients.[44] Furthermore, presence of tumor-infiltrating T_{reg} cells correlated with a worse prognosis in patients with ovarian cancer and renal cell carcinoma.[45,46] These findings confirm the active role for infiltrating immune cells in controlling cancer progression. Moreover, depletion of T cells in an osteosarcoma mouse model and in the Swarm rat chondrosarcoma model resulted in a markedly reduced survival rate and an accelerated growth rate, respectively, revealing a function for $CD8^+$ T cells in this experimental setting in slowing sarcoma progression.[47,48] Although a high number of infiltrating $CD8^+$ T cells does not seem to be a clear prognostic marker for survival in patients with soft-tissue sarcoma, high expression of PD-1 on TILs and expression of PD-L1 correlate with worse survival rates in these patients, suggesting that expression of PD-1 and PD-L1 in soft-tissue sarcomas could inhibit T-cell-mediated control of cancer progression.[49,50]

Mapping of the T-cell infiltrate in the tumor microenvironment and its association with patient survival has been investigated in some specific sarcoma subtypes (Table 1). A high number of tumor-infiltrating $CD8^+$ T cells correlated with improved overall survival in Ewing sarcoma.[51] In osteosarcoma, PD-L1 expression on tumor cells associated with higher numbers of TILs and poorer survival rate, indicating that the PD-1/PD-L1 axis is an important immune evasion strategy of sarcomas.[52] PD-L1 expression has also been observed in almost 50% of dedifferentiated chondrosarcomas and correlated with a higher number of TILs and positive HLA class I expression in tumor cells, providing a rationale for using anti-PD-1 and anti-PD-L1 treatment in this sarcoma subtype.[53] A high number of intratumoral lymphocytes was also observed in EBV-associated leiomyosarcomas and inflammatory myofibroblastic tumors.[54,55]

Table 1 Examples of association between TILs and survival in some sarcoma types

Sarcoma type	Type of immune infiltrate	Effect on survival	Reference
Angiosarcoma	$CD8^+$ T cells	Improved	96
Gastrointestinal stromal tumors	$CD3^+$ T cells, NK cells	Improved	97
Ewing sarcoma	$CD8^+$ T cells	Improved	51
Dedifferentiated liposarcoma	Tertiary lymphoid structures	Poor	98
Malignant peripheral nerve sheath tumor	$CD8^+$ T cells	No effect	99
Dedifferentiated chondrosarcoma	$CD3^+$ T cells	No effect	53
Osteosarcoma	$CD8^+$/$FOXP3^+$ T cells	Improved	100
	$CD3^+$ T cells	No effect	101

Table 2 Reported PD-L1 expression in some sarcoma subtypes

Sarcoma subtype	Positive cases (%)	Reference
Angiosarcoma	50–80	50,65
Chondrosarcoma	41–75	53,65
Ewing sarcoma	29–67	50,65,69
Leiomyosarcoma	32–70	50,65
Malignant peripheral nerve sheath tumor	17–67	50,65,99
Osteosarcoma	28–57	65,101
Rhabdomyosarcoma	38–63	65,69
Synovial sarcoma	25–75	50,65,69
Dedifferentiated liposarcoma	67–82	50,65
Gastrointestinal stromal tumor	29	102

Pollack *et al*[56] used gene expression profiling to measure the amount of T-cell infiltration and found a higher degree of T-cell infiltration in undifferentiated pleomorphic sarcomas and leiomyosarcomas, and a low degree in synovial sarcomas. Interestingly, they also showed that the degree of T-cell infiltration and clonality significantly correlated with PD-1 and PD-L1 expression in all investigated sarcoma subtypes, suggesting that sarcomas with a high number of TILs might be more suitable for anti-PD-1 and anti-PD-L1 therapy than sarcomas with a low degree of T-cell infiltration.

Expression of PD-1 and PD-L1

Expression of PD-1 and PD-L1 in the tumor microenvironment might be an important predictive biomarker for anti-PD-1 therapies. In one of the first clinical trials with anti-PD-1 antibodies, tumor samples were immunohistochemically stained for PD-L1 before treatment. Thirty-six percent of the patients with PD-L1-positive tumors had an objective response while none of the patients with PD-L1-negative tumors responded to therapy.[6] However, in a large cohort of patients with advanced melanoma, patients with a PD-L1-positive tumor showed an objective response rate of 57.5% to treatment with an anti-PD-1 antibody, whereas patients with a PD-L1-negative tumor showed an objective response rate of 41.3%.[57] These findings suggest that PD-L1 expression might not always be a clear-cut biomarker for the response to anti-PD-1 therapy.

PD-L1 can be overexpressed in cancer cells as result of genetic alterations. In diffuse large B-cell lymphomas, the *PD-L1* gene was shown to be translocated and placed under the regulation of a different promotor, notably the immunoglobulin heavy-chain locus, leading to upregulation of PD-L1 expression.[29] Loss of PTEN in colorectal cancer was also shown to result in increased expression of PD-L1.[58] Interestingly, some soft-tissue sarcomas, such as liposarcomas and leiomyosarcomas, harbor genetic mutations in *PTEN* and loss of PTEN has recently be shown to associate with resistance to anti-PD-1 therapy in metastatic uterine leiomyosarcoma.[59–64]

As already discussed above, the expression of PD-L1 is a frequently observed immune evasion strategy of certain sarcomas. So far, several studies have assessed PD-L1 expression in different sarcoma subtypes using immunohistochemistry (Table 2). In a large analysis of over 2000 sarcomas, ~50% of all sarcomas displayed expression of PD-L1 with immunohistochemistry. Notably, PD-L1 expression was observed in leiomyosarcomas, chondrosarcomas, liposarcomas, and undifferentiated pleomorphic sarcoma using immunohistochemistry with an anti-PD-L1 antibody.[65] Frequent expression of PD-1 and PD-L1 was also observed in synovial sarcoma and angiosarcoma.[50] In a cohort of 38 osteosarcoma tumor specimens PD-L1 expression was detected with RT-PCR in 32 cases of which 9 had a relative high PD-L1 mRNA expression level.[66] In HHV8-associated Kaposi sarcoma, PD-L1 expression has also been demonstrated in a large subset of cases.[67,68] Another study could detect PD-L1 expression in the dedifferentiated chondrosarcoma subtype, but not in the conventional, clear cell or mesenchymal chondrosarcoma subtype.[53]

Although these studies provide a rough estimate of the percentage of PD-L1-expressing sarcomas, they also highlight some discrepancies.[50,65,69] This lack of reproducibility can be partially explained by the small number of cases and variable cutoff points for PD-L1-positivity. Moreover, the difference could also be explained by different types of antibodies used for the immunohistochemistry of PD-L1. Therefore, assessment of PD-L1 expression in sarcomas with immunohistochemistry is not, so far, a reliable predictive biomarker to preselect patients for treatment with immune checkpoint inhibitors and efforts should be made to standardize this procedure.

Mutational Load

Next-generation sequencing technologies have made it possible to comprehensively detect somatic mutations in individual tumors and to reveal mutational signature profiles. These techniques therefore provide a novel powerful tool to unravel the underlying genetic pathogenesis of sarcomas. The mutational burden of a tumor can be predictive for the outcome of treatment with immune checkpoint inhibitors. Snyder *et al*.[70] showed that treatment with ipilimumab, an anti-CTLA-4 antibody, was significantly more effective in patients with melanomas carrying more than 100 mutations per coding genome when compared to patients with <100 mutations in their tumors. For non-small-cell lung cancer, patients with a high nonsynonymous mutation burden were also more likely to show improved clinical benefit when treated with an anti-PD-1 antibody than patients with a low nonsynonymous mutation burden.[71] These findings support that a higher mutational load probably translates into an increased probability that neoantigens are recognized by the immune system. In line with these findings, the association

Table 3 Examples of mutational load in different sarcoma types reported by sequencing studies

Sarcoma type	Reported mutations	Mutations/Mbp	Determined mutation rate	Reference
Ewing sarcoma	6	0.15	Average in protein-coding sequences	78
Osteosarcoma	5–103	1.15	Mean mutation rate, whole genome	77
Solitary fibrous tumors	12–41	0.66	Median mutation rate, whole exome	103
Angiosarcoma	—	0.7–2.2	Whole genome	104
Uterine leiomyosarcoma	240–779	—	Exome	80
Malignant peripheral nerve sheath tumor	14–208	—	Whole genome	105
Chondrosarcoma	1–115	—	Exome	81
Rhabdomyosarcoma	24	—	Whole exome	82
Well-differentiated liposarcoma	16–71	—	Whole exome	85
Dedifferentiated liposarcoma	24–56	—	Whole exome	85
Myxoid liposarcoma	15–33	—	Whole exome	85

between response to ipilimumab treatment, overall mutational, and neoantigen load has been shown in melanoma patients.[72,73]

Although the application of next-generation sequencing in sarcomas is a developing research field, recent studies suggest that some sarcomas are driven by an intermediate mutational load (Table 3). For example, a high median frequency of somatic mutations of ~14 mutations per megabase pair (Mbp) has been reported for melanoma and a low median frequency of 0.37 mutations per Mbp as reported for acute myeloid leukemia. Compared with these reported mutation rates, the mean mutation rate of 1.15 mutations per Mbp found in a cohort of 20 high-grade intramedullary osteosarcomas is an intermediate rate, which is—although difficult to compare, as often different pipelines for analysis have been utilized—roughly similar to the median mutational rate of breast cancer shown by Alexandrov and colleagues.[74–77] Furthermore, this cohort of osteosarcomas showed a range in the mutational rate between 0.49 and 3.99 mutations per Mbp, suggesting that some individual cases could benefit more from treatment with immune checkpoint inhibitors due to their higher-than-average mutational rate.[77] Ewing sarcoma has a reported mutation rate of 0.15 mutations per Mbp of coding sequence in one study and a mutation rate of 0.62 per Mbp in another study, both indicating that the mutational rate of this translocation-driven tumor can be categorized with other cancer types with a low mutational rate.[75,78,79] Exome sequencing of uterine leiomyosarcomas revealed a mean of 373 somatic mutations per sample, whereas the mutation burden in chondrosarcoma has been shown to range from 1 to 115 somatic mutations.[80,81] In another study, exome sequencing of rhabdomyosarcoma cases revealed a mean number of mutations of 24.0 mutations per sample in a primary tumor, while metastatic tumors showed 43.3 mutations per sample and relapsed tumors 42.0 mutations

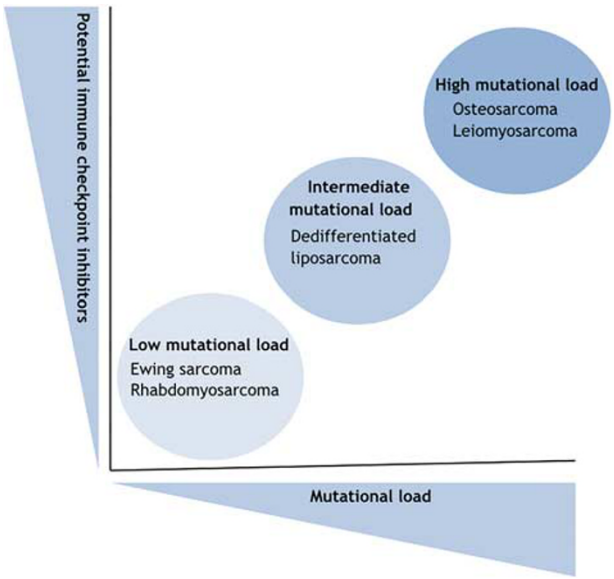

Figure 3 Mutational load and potential for immune checkpoint inhibitors in sarcomas. Some sarcomas such as Ewing sarcoma are mainly driven by specific translocations and do not display a high mutational profile, while other sarcomas such as osteosarcoma show a higher mutation burden and a more pleomorphic histology. A higher mutational load increases the change of neoantigen formation and enhances the immunogenicity of the tumor.

per sample, although these differences were not statistically significant.[82] Epithelioid sarcomas have a coding somatic point mutation rate similar to that of ovarian carcinoma, indicating a relevant mutation rate in this sarcoma type, which could be beneficial for treatment with immune checkpoint inhibitors.[83,84] In 12 liposarcoma specimens of different subtypes, a total of 377 potential somatic mutations were detected of which 91% was validated with Sanger sequencing.[85] Although some next-generation sequencing

studies have reported the mutation burden of certain sarcomas, it is still very difficult to directly compare the mutational load of different sarcomas based on these studies. However, as the field of next-generation sequencing is rapidly developing, the mutational load of more sarcomas can soon be unraveled and compared with other cancers, which will be a crucial step in further exploring the potential of treatment with immune checkpoint inhibitors in sarcomas (Figure 3).

DNA Mismatch Repair System

The proteins of the DNA mismatch repair (MMR) system are crucial in restoring incorporated mismatched bases during replication. As such, MMR-deficient tumors have been shown to be genetically instable tumors with a relative higher mutational load. For example, MMR-deficient colorectal cancers often show a high mutational rate, microsatellite instability, and a higher degree of TILs, suggesting an ongoing immune response against the tumor.[86,87] The higher degree of tumor infiltration by lymphocytes and the higher mutational load suggest that MMR-deficient tumors could be more suitable for the treatment with immune checkpoint inhibitors than MMR-proficient tumors. In a phase 2 clinical trial published by Le *et al*,[8] 41 patients with metastatic carcinoma were treated with an anti-PD-1 antibody, and in line with their hypothesis, they found a significantly increased progression-free survival rate and overall survival in patients with MMR-deficient colorectal cancer compared to patients with MMR-proficient colorectal cancer.

The outcome of this clinical trial provides clear evidence for the use of immune checkpoint inhibitors in MMR-deficient cancers. Although evidence is often conflicting, in some cases, certain sarcomas can display defects in the MMR system.[88] Twenty-one percent of the cases in a cohort of uterine carcinosarcomas were shown to have a defective MMR system based on a microsatellite instability phenotype and a small percentage of leiomyosarcomas are also deficient in MMR.[89,90] Ongoing research could shed more light on MMR deficiency in sarcomas, as its role still remains to be elucidated.

Biomarkers for Hyperprogression

Very recently, the phenomenon of hyperprogression was described in cohorts of patients that were treated with anti-PD-1/PD-L1 and anti-CTLA-4 antibodies.[91,92] In some of these patients, treatment with these immune checkpoint inhibitors accelerated the growth of their tumors. This hyperprogression was shown to correlate with amplification of the *MDM2* and *MDM4* genes and mutations in the *EGFR* gene.[92] In a large survey for *MDM2* amplification in a variety of tumor types, *MDM2* amplification was found to be most prevalent in sarcomas.[93] Amplification of the 12q13-15 region, including the *MDM2* gene is the hallmark of well-differentiated and dedifferentiated liposarcoma, as well as parosteal osteosarcoma and is used as diagnostic marker.[94] In addition, other sarcomas also display MDM2 amplification in a low percentage of the tumors, including conventional osteosarcomas and malignant peripheral nerve sheath tumors.[95] These studies suggest that for these sarcoma subtypes treatment with immune checkpoint inhibitors must be carefully considered.

CONCLUSIONS

Immune checkpoint inhibitors have accelerated the immunotherapy revolution in oncology. As metastatic sarcomas have limited options for treatment, these therapeutics could be an interesting novel treatment option. While some first promising results in sarcoma are now being published, several clinical trials are still ongoing. A selection of sarcomas that are most suitable for treatment with immune checkpoint inhibitors can be guided by recently proposed biomarkers in other cancers. As a correlation between TILs and PD-L1 has been found in certain sarcomas, such as osteosarcoma and dedifferentiated chondrosarcoma, PD-L1 expression in these sarcomas can be considered an immunosuppressive tool to prevent TILs from eliminating tumor cells. This provides a strong rationale for therapy with anti-PD-1 or anti-PD-L1 antibodies in sarcomas with a high degree of TILs or PD-L1 expression. Furthermore, some sarcomas, such as osteosarcoma and epithelioid sarcomas, show an intermediate mutation burden when compared with other cancer types. In addition, although some sarcomas display a higher mutational load than others, individual cases with a relatively higher mutational load can often be identified in a cohort of patients. As a high mutation burden has been associated with a higher neoantigen load and a better survival rate after administration of immune checkpoint inhibitors, patients with a hypermutated sarcoma could benefit from treatment with immune checkpoint inhibitors. Larger exome-wide and genome-wide sequencing studies could provide novel insights in the mutational landscape of sarcomas and can help guiding the selection of sarcoma types for treatment with immune checkpoint inhibitors.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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