Molecules and Cells

Minireview

Sarcoma Immunotherapy: Confronting Present Hurdles and Unveiling Upcoming Opportunities

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Sarcomas are rare and heterogeneous mesenchymal neoplasms originating from the bone or soft tissues, which pose significant treatment challenges. The current standard treatment for sarcomas consists of surgical resection, often combined with chemo- and radiotherapy; however, local recurrence and metastasis remain significant concerns. Although immunotherapy has demonstrated promise in improving long-term survival rates for certain cancers, sarcomas are generally considered to be relatively less immunogenic than other tumors, presenting substantial challenges for effective immunotherapy. In this review, we examine the possible opportunities for sarcoma immunotherapy, noting cancer testis antigens expressed in sarcomas. We then cover the current status of immunotherapies in sarcomas, including progress in cancer vaccines, immune checkpoint inhibitors, and adoptive cellular therapy and their potential in combating these tumors. Furthermore, we discuss the limitations of immunotherapies in sarcomas, including a low tumor mutation burden and immunosuppressive tumor microenvironment, and explore potential strategies to tackle the immunosuppressive barriers in therapeutic interventions, shedding light on the development of effective and personalized treatments for sarcomas. Overall, this review provides a comprehensive overview of the current status and potential of immunotherapies in sarcoma treatment, highlighting the challenges and opportunities for developing effective therapies to improve the outcomes of patients with these rare malignancies.

Keywords: bone cancer, immunotherapy, sarcomas, soft tissue sarcoma, tumor microenviorment

INTRODUCTION

Sarcomas represent a heterogeneous group of mesenchymal neoplasms originating from the bone or soft tissues such as the cartilage, muscle, and other connective tissues. Sarcomas account for 15% of childhood and 1% of all adult malignancies. Despite being a rare malignancy, approximately 13,190 patients in the United States were diagnosed with sarcoma in

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2022, with 5,130 fatalities (Siegel et al., 2022). Patients with sarcomas experience a high probability of local recurrence (5-year local recurrence rate of 19%-51%) (Pawlik et al., 2006; Thorkildsen et al., 2021; Xie et al., 2015; Zhao et al., 2018), and repeated relapses eventually accompany distant metastasis (Panagi et al., 2022). Sarcomas are classified into soft tissue sarcomas (STSs), including rhabdomyosarcoma and liposarcoma, and bone sarcomas (BSs), including osteosarcoma and chondrosarcoma, encompassing more than 70 distinct subtypes (Hui, 2016). The main treatment challenge to overcome is the propensity for local recurrence or eventual metastatic spread despite extensive adjuvant therapy (Goorin et al., 1984; Patel et al., 2017; von Konow et al., 2021), which is related to the substantial heterogeneity of these tumors, leading to varied symptoms, treatment responses, and prognoses among patients (Du et al., 2020; Grunewald et al., 2020). Delayed diagnosis is common, with symptoms in the pediatric population often misdiagnosed as growing pains, further narrowing the available treatment options (Lodhia et al., 2022).

Surgical removal is the standard treatment approach for localized, clinically resectable STSs and BSs (Blasius et al., 2022; Gronchi, 2021). Radiotherapy can also be administered in neoadjuvant, adjuvant, or definitive settings (Hoefkens et al., 2016), although the optimal approach varies depending on individual cases. Chemotherapy is recommended for patients with high-grade STSs following resection, but has failed to demonstrate distinct improvements in terms of relapse-free survival or overall survival (OS) (Squires et al., 2020). Although BS subtypes generally do not respond well to chemotherapy, multidrug chemotherapy has shown moderate treatment efficacy for osteosarcoma and Ewing sarcoma in combination with surgery (Ingley et al., 2022). Chondrosarcoma, another common BS subtype, is generally refractory to conventional chemotherapy and radiation therapy, with no supporting efficacy data available (Gelderblom et al., 2008; Monga et al., 2020; Riedel et al., 2009). Metastatic sarcomas exhibit substantial resistance to chemo- and radiotherapy (Olivier et al., 2015). Although the exact mechanisms underlying chemotherapy resistance remain elusive, emerging evidence implicates epigenetic (Rytlewski et al., 2022) and genetic modifications (Kim et al., 2020), and epithelial-mesenchymal transition (EMT) plasticity (Sannino et al., 2017), and the tumor microenvironment (TME) (Tu et al., 2016) as contributory factors. Sarcomas exhibit radioresistance, often attributed to epigenetic changes (de Jong et al., 2019), non-coding RNAs (Chen et al., 2022), and slow cell division rates (Chen et al., 2022; Li et al., 2014). Collectively, current adjuvant chemo- and radiotherapies are insufficient in preventing the metastasis and recurrence of sarcomas, and there remains uncertainty regarding the best resection margins and appropriate use of chemo- and radiotherapy (Hoefkens et al., 2016; Zhang et al., 2022).

Immunotherapy, a strategy that modulates the immune system to target cancer cells, has demonstrated promise for several cancer types (Tan et al., 2020; Waldman et al., 2020), with significantly improved long-term survival rates in melanoma and lymphomas, eliciting less cytotoxicity compared to traditional chemotherapies (Abramson et al., 2020; Tan et al., 2020). Notably, immunotherapy can achieve simultaneous local and distant control while adapting to changes in antigen expression over time, which is significant for preventing recurrent and metastatic tumors (Finkelstein et al., 2012). Cancer immunotherapy relies on two main immune mechanisms: immune surveillance and immune editing. Immune surveillance involves the detection and elimination of tumors by the immune system (Zitvogel et al., 2006), whereas immune editing modifies tumor immunogenicity to either strengthen the immune system's capacity to eliminate tumor cells or prevent the emergence of immune-resistant tumor variants (O'Donnell et al., 2019). By selectively manipulating the anti-tumor immune mechanism, immunotherapy has potential to prevent cancer metastasis and recurrence (Edwards et al., 2021).

Several successful cancer immunotherapies have emerged recently utilizing cancer vaccines, adoptive transfer therapy, or cytokine therapy (Zhang and Zhang, 2020). However, sarcomas are considered to be relatively less immunogenic than other cancers (Rytlewski et al., 2021; Weng et al., 2022; Zhu et al., 2020). Specifically, sarcomas lack well-established antigens and often display an immunosuppressive TME. Therefore, understanding the immune escape mechanisms and immunosuppressive TME in sarcoma is essential for developing effective sarcoma immunotherapy. In this review, we provide an update of the current status of sarcoma immunotherapy, focusing on the needs, emerging strategies, and challenges.

IMMUNOTHERAPIES: ADDRESSING UNMET NEEDS FOR SARCOMA TREATMENT

Cancer testis antigen-based vaccine therapy

Sarcomas typically express cancer testis antigens (CTAs), a group of tumor-associated antigens found predominantly in male germ cells in the testis but not in the adult somatic tissue. CTAs are generally quiescent in healthy tissues, but their expression is induced in various malignancies (Whitehurst, 2014). The epitopes of CTAs can be recognized by T cells, facilitating immune activation (Juretic et al., 2003).

Among the several CTAs identified in sarcomas to date, New York esophageal squamous cell carcinoma 1 (NY-ESO-1), melanoma-associated antigen (MAGE), and preferentially expressed antigen of melanoma (PRAME) are the most prevalent (Kakimoto et al., 2019; Wei et al., 2020). NY-ESO-1 is detected in various sarcoma subtypes, including myxoid liposarcomas, osteosarcomas, and synovial sarcomas (Hashimoto et al., 2022; Jungbluth et al., 2001; Kakimoto et al., 2019). MAGE expression has been observed in osteosarcoma, synovial sarcoma, and myxoid/round cell liposarcoma, with distinct MAGE subtypes exhibiting variable expression levels. For instance, osteosarcoma exhibited high expression of MAGE-A1, -A2, and -A3, but low expression of MAGE-A12 (Zou et al., 2012). Synovial sarcoma and myxoid/round cell liposarcoma displayed elevated levels of MAGE-A4 (Hemminger et al., 2014; Iura et al., 2017). PRAME expression is elevated in osteosarcoma, synovial sarcoma, and myxoid/round cell liposarcoma (Epping et al., 2005; Wei et al., 2020).

The potential of CTAs as tumor vaccines has been explored

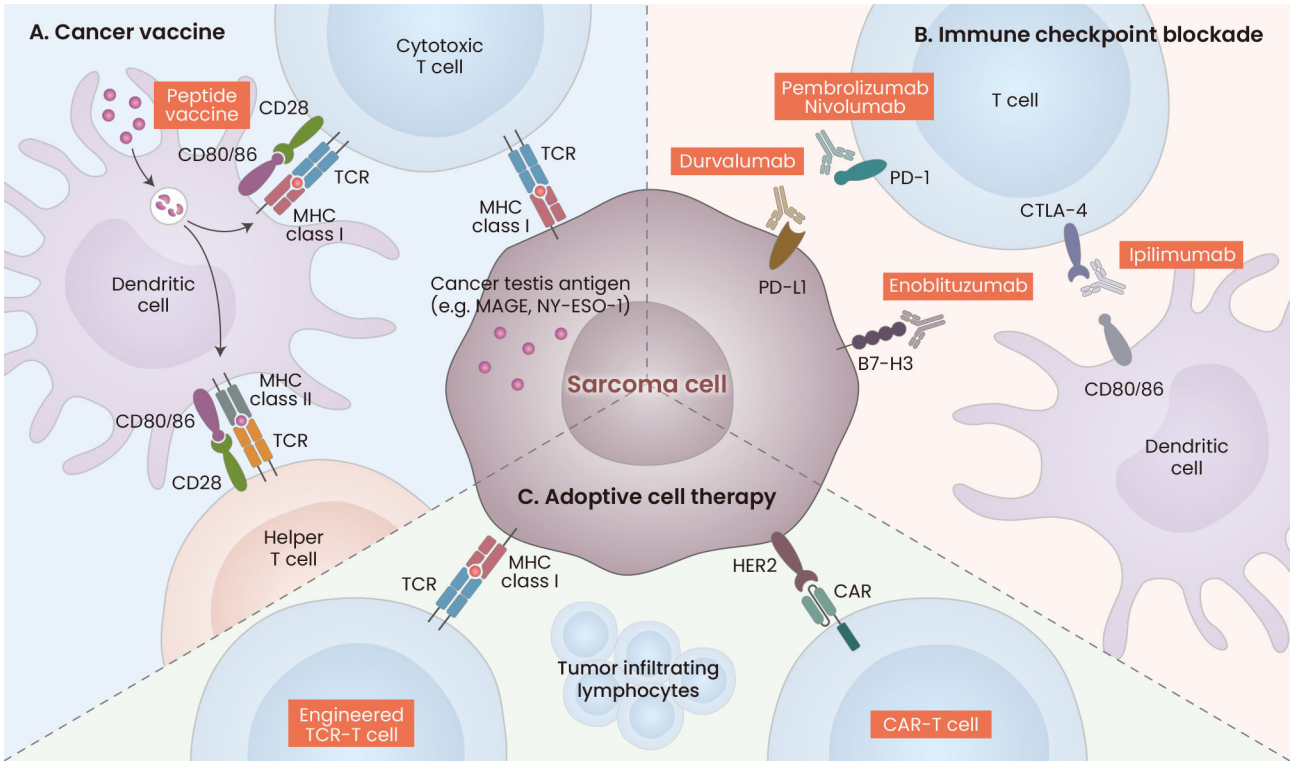

Fig. 1. Current immunotherapies used for sarcoma treatment. (A) Cancer vaccine: Dendritic cells present sarcoma-derived antigen or neoantigen peptides through MHC class II to T cell receptor (TCR), activating T cells with CD28-CD80/86 co-stimulation. MHC class I presents tumor-specific antigens such as MAGE-A1 or NY-ESO-1, leading to cytotoxic T-lymphocyte (CTL)-mediated killing of sarcoma cells. (B) Immune checkpoint blockade: Sarcoma cells evade T cell-mediated cytotoxicity by expressing immune checkpoint ligands such as programmed cell death ligand 1 (PD-L1), which suppresses T cell activation by binding with complementary receptors on T cells such as programmed cell death 1 (PD-1). Immune checkpoint blockade antibodies targeting PD-L1 or PD-1 interfere with this interaction. Similarly, blocking the cytotoxic T lymphocyte associated antigen 4 (CTLA-4) receptor binding with CD80/86 complex prevents CTL suppression. (C) Adoptive cell therapy: Engineered TCR-T and chimeric antigen receptor (CAR)-T cells specifically engage with tumor cells that express the target antigen on their surface. CAR-T cells are designed to bind with sarcoma cells independent of MHC class I complex. MAGE, melanoma-associated antigen; NY-ESO-1, New York esophageal squamous cell carcinoma 1; HER2, human epidermal growth factor receptor 2.

with the aim of enhancing immunological recognition and improving immune responses against tumors (Fig. 1A). Tumor vaccines can be classified into two distinct categories: peptide-based vaccines, which involve the direct delivery of antigens, and dendritic cell (DC)-based vaccines, which employ antigen-presenting DCs for targeted immunotherapy (Saxena et al., 2021; Tagliamonte et al., 2014). NY-ESO-1 peptide was successfully delivered using a *Salmonella typhimurium* vaccine strain, eliciting NY-ESO-1-specific $CD8^+$ and $CD4^+$ T cells in peripheral blood mononuclear cells from patients (Nishikawa et al., 2006). Treatment of another NY-ESO-1 vaccine, CDX-1401, induced both humoral and cellular immunity to NY-ESO-1 in 45 patients with multiple malignant tumors, including sarcomas, 13 of whom experienced disease stabilization and two of whom exhibited tumor regression (Dhodapkar et al., 2014). This first-in-human study of DC-based vaccines showed promise in treating sarcomas. However, the application of CTA-targeting vaccines in sarcomas has yielded inadequate results compared to that for other malignant tumors. Possible explanations for this discrepancy include low T cell functional avidity, high regulatory T cell (T_{reg}) activity, and higher expression of immune checkpoints such as programmed cell death 1 (PD-1) in sarcomas (Wei et al., 2020).

Immune checkpoint inhibitors

The expression of immune checkpoint proteins such as PD-1 and its ligand (PD-L1), as well as cytotoxic T lymphocyte associated antigen 4 (CTLA-4), is a key immune evasion mechanism of tumors (Beatty and Gladney, 2015; Lee et al., 2021b). Large cohort studies demonstrated the presence of PD-1 (43% of sarcoma cases) (Kim et al., 2016) and CTLA-4 (32% of sarcoma cases) on T cells, as well as PD-L1 (23% of cases) on tumor cells in various STSs and BSs (Paydas et al., 2016; Samiei et al., 2022; Zheng et al., 2015). These findings underscore the potential of these checkpoint proteins as emerging therapeutic targets for immunotherapies in sarcoma treatment (Fig. 1B).

To date, clinical trials evaluating the efficacy of immune checkpoint inhibitors (ICIs) in patients with STSs have demon-

strated modest results, with varying responses depending on treatment regimens or histological subtypes. Pembrolizumab, a PD-1 monoclonal antibody, was assessed in a phase II trial involving STSs and BSs, yielding meaningful results in undifferentiated pleomorphic sarcoma (objective response in 40% of patients) and liposarcoma (objective response in 20% of patients), while demonstrating limited efficacy in BS (Tawbi et al., 2017). Additionally, pembrolizumab exhibited high-level, prolonged anti-tumor activity in select sarcoma histotypes such as alveolar soft part sarcoma and chordoma, with 6-month OS rates of 100% and 75%, respectively (NCT03012620). Ipilimumab, a CTLA-4 antibody, and nivolumab, a PD-1 antibody, are two other notable immune checkpoint blockade antibodies promoting anti-tumor immunity in sarcoma (Tawbi et al., 2017; Weber, 2007). Nivolumab resulted in a complete or partial response in 5% of patients with advanced sarcoma when delivered as a monotherapy, whereas ipilimumab monotherapy resulted in a 7% partial response rate; their combination displayed promising efficacy with three times higher confirmed objective responses (16%) in multiple sarcoma subtypes (D'Angelo et al., 2018). A recent clinical trial demonstrated a progression-free survival (PFS) rate of 49% at 12 weeks in patients with various advanced or metastatic sarcomas that received combined therapy with durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA-4) (Somaiah et al., 2022).

Adoptive cellular therapy

Immune evasion in cancer can arise from a lack of neo-antigens or defects in antigen presentation. Adoptive cellular therapy (ACT), or cellular immunotherapy, has potential to surmount these challenges by modifying patient-specific T cells to enhance the targeting of tumor-specific antigens. ACT is a personalized treatment approach involving the isolation, *ex vivo* modification, expansion, and reinfusion of a patient's own immune cells, thus bypassing antigen presentation defects (Zhao and Cao, 2019). Various forms of ACT exist, including engineered T cell receptor (TCR) and chimeric antigen receptor (CAR) T cell therapy (June et al., 2018) (Fig. 1C).

TCR therapies using Afamitresgene autoleucel (afami-cel) and Letetresgene autoleucel (lete-cel) are currently under assessment in a phase I/II trial to induce anti-tumor responses in patients with synovial sarcoma and myxoid/round cell liposarcoma. These therapies consist of autologous $CD4^+$ and $CD8^+$ T cells that are genetically modified to express a TCR recognizing MAGE-A4 (afami-cel) or NY-ESO1 (lete-cel) bound to human leukocyte antigen A02. Pooled data from 69 patients (phase I, n = 18; phase II, n = 51) showed an investigator-assessed objective response rate of 36.2% (40.7% in synovial sarcoma; 10.0% in myxoid/round cell liposarcoma) (D'Angelo et al., 2022).

Human epidermal growth factor receptor 2 (HER2) is a potent antigen identified in various sarcomas, albeit with low expression (Ahmed et al., 2009; Yan et al., 2015). CAR-T therapy targeting HER2 exhibited tumor regression in both localized and metastatic lesions, with prolonged survival in mice inoculated even with the low-HER2-expressing osteosarcoma cell line (Ahmed et al., 2009). In a clinical phase I/II study of HER2 CAR-T cells to treat HER2-positive osteosarcoma, Ewing sarcoma, neuroectodermal tumor, and desmoplastic small round cell tumor, 4 of the 17 patients experienced stable disease for 12 weeks to 14 months and three patients had their residual tumors removed without additional therapy, with one specimen achieving ≥90% tumor necrosis (Ahmed et al., 2015). Combination of this therapy with lympho-depleting chemotherapy showed further improvement in outcomes (Navai et al., 2019). The ganglioside antigen GD2 is expressed in Ewing sarcoma and osteosarcoma (Kailayangiri et al., 2012; Roth et al., 2014). GD2-specific CAR-T cells in combination with AMG102, a human growth factor receptor neutralizing antibody, inhibited the primary tumor growth and metastasis of Ewing sarcoma (Charan et al., 2020).

TACKLING IMMUNOSUPPRESSIVE BARRIERS IN THERAPEUTIC INTERVENTIONS FOR SARCOMA

Immunotherapy has shown beneficial results in treating various cancers, yet its efficacy in STSs and BSs remains restricted to specific subtypes (Ayodele and Razak, 2020; Panagi et al., 2022), necessitating further strategies to improve efficacy (Klemen et al., 2021). Some combined immunotherapies, in conjunction with chemotherapy, radiotherapy, and targeted therapies, have demonstrated improved prognosis in sarcomas (D'Angelo et al., 2018; Husain et al., 2023; Kelly et al., 2020; Tang et al., 2021). However, challenges persist in treating immunologically "cold" sarcomas. In this section, we discuss key mechanisms that might contribute to these challenges and the approaches developed to potentially overcome them (Fig. 2).

Low tumor mutation burden

Tumor mutation burden (TMB), reflecting the number of mutations harbored by tumors, is a valuable biomarker positively related to the immunotherapy response (Strickler et al., 2021). Among 151 immunotherapy-treated patients, a higher TMB correlated with better outcomes, including response rate, PFS, and OS (Goodman et al., 2017). This favorable response is attributed to a positive correlation between TMB and immunogenic neoantigens (Lagos et al., 2020).

The U.S. Food and Drug Administration approved pembrolizumab for cancers with a TMB ≥ 10 mutations/Mb, indicating therapeutic implications based on qualitative next-generation sequencing; however, no patients with sarcoma were enrolled in this study (Marabelle et al., 2020). According to a comprehensive mutational analysis pipeline that incorporated clinical data, it was found that among 206 cases of STS, patients exhibited an average TMB of 1.06 mutations/Mb (Cancer Genome Atlas Research Network, 2017). In the case of BSs, TMB ranged from 0 to 7 mutations/Mb among 31 osteosarcoma patients. The 3rd quartile of TMB in this patient cohort was determined to be 2.565 mutations/Mb (Xie et al., 2021). Collectively, sarcomas are primarily characterized by low TMB (compared to melanoma and lung cancers, with 20% of patients meeting the TMB cutoff of ≥10 mutations/Mb for pembrolizumab), rendering them immunologically cold tumors (Doig et al., 2022). This poses a considerable challenge for the development of effective immunotherapies

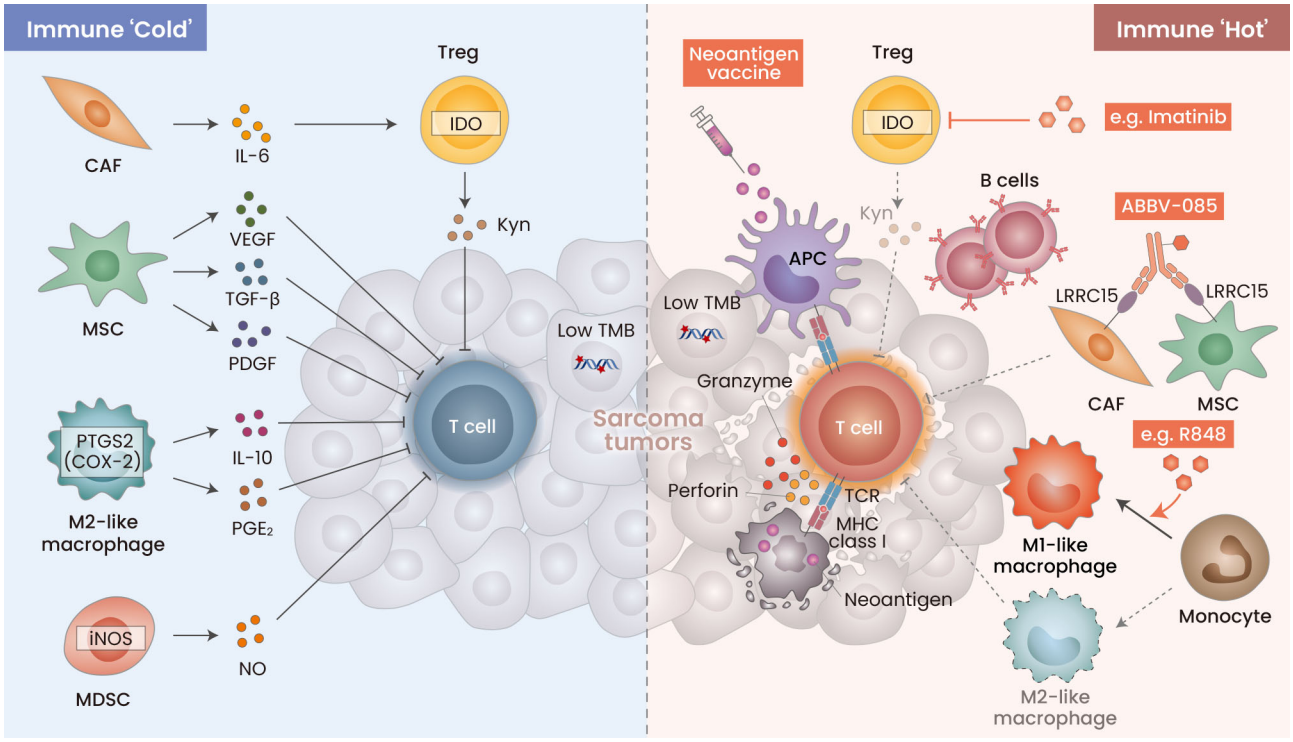

Fig. 2. Barriers to immunotherapy in sarcomas and strategies for modulating their tumor microenvironment. Sarcomas are primarily recognized by their low tumor mutation burden, which classifies them as tumors displaying limited immune reactivity. The immunosuppressive features characterizing the tumor microenvironment (TME) of sarcomas are correlated with the existence of regulatory T cells, tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSCs) (left). These components present prospective targets for crafting immunotherapy approaches with enhanced efficacy in tackling sarcomas (right). A personalized cancer vacccine addresses low tumor mutation burden by utilizing neoantigens to activate precise cytotoxic T cells targeting sarcoma tumors. TAMs can be regulated by inhibiting M2-like TAMs or by stimulating M1-like macrophages to amplify anti-tumor immune responses. Approaches that combine imatinib with CTLA-4 blockade or employ anti-CD25 antibodies alongside PD-1 blockade can control regulatory T cells (T_{regs}) and improve treatment outcomes. CCR4 inhibitors have shown potential in reducing T_{reg} infiltration. Cancer-associated fibroblasts (CAFs) expressing LRRC15 have emerged as promising targets, with the antibody-drug conjugate ABBV-085, which targets LRRC15, displaying notable anti-tumor activity in clinical trials. These strategies seek to overcome the immunologically cold TME in sarcomas, enhancing the efficacy of immunotherapy. MSCs, mesenchymal stem cells; IDO, indoleamine 2,3-dioxygenase; TMB, tumor mutation burden; TCR, T cell receptor.

for sarcoma patients.

To overcome the challenge of low TMB, recent strides in cancer immunotherapy have explored the capacity of neoantigens to stimulate cytotoxic T cells, targeting sarcoma tumor cells with distinct specificity (Gubin et al., 2014; Yang et al., 2019). Originating from somatic mutations or chromosomal rearrangements, neoantigens are frequently present in sarcomas. In synovial sarcomas, peptides from the typical SYT-SSX fusion have been effective in generating specific CTLs for synovial sarcoma (Kawaguchi et al., 2012; Sato et al., 2002). The implementation of a customized immunotherapy strategy, tailored to the distinctive tumor traits of each individual, presents exciting new opportunities for effectively treating sarcomas with low TMB.

Immunosuppressive TME

The TME plays a pivotal role in shaping the immune response to cancer cells; thus, understanding its composition is essential for developing effective immunotherapies. Petitprez et al. (2020) demonstrated that STSs with immune-deprived TMEs displayed low expression of immune checkpoint-related genes (*PD-1*, *PD-L2*, *CTLA-4*, and *TIM3*). In contrast, B-cell-rich tertiary lymphoid structures, predominantly observed in immune-rich classes, were positively correlated with improved survival and higher response rates to PD-1 blockade. The immune cell distribution within the TME critically influences the immune response in sarcomas. Tight-junction protein 1 (TJP1) may play a role in shaping the immunosuppressive landscape of TME in leiomyosarcoma. TJP1 contributes to tumor growth by modulating cell-cell aggregation and cytokine-mediated communication in TME. Notably, TJP1 exhibits a negative correlation with expression of colony stimulating factor 1 (CSF1), which typically fosters an immunosuppressive TME through macrophage regulation (Lee et al., 2021a). Only 22% and 12% of 50 sarcoma specimens exhibited PD-1 and PD-L1 expression, respectively, whereas 75% of the specimens contained $FOXP3^+$ T_{regs} in the immunological milieu (D'Angelo et al., 2015). Single-cell transcriptomic pro-

filing of osteosarcomas and their TMEs identified 11 main cell clusters such as osteoblastic cells, myeloid cells, mesenchymal stem cells (MSCs), fibroblasts, and tumor-infiltrating lymphocytes (TILs). Notably, the TILs cluster displayed high expression of T cell exhaustion inhibitory receptors, including TIGIT and LAG3, and blocking TIGIT enhanced the cytotoxic effects of $CD3^+$ T cells against osteosarcoma cells (Zhou et al., 2020).

Antigen presentation by tumor-associated macrophages (TAMs) contributes to anti-tumor immune responses. M1-like macrophages exhibit anti-tumoral functions, whereas M2-like macrophages exhibit pro-tumoral functions. Immunohistochemistry of STS samples demonstrated the widespread presence of macrophages in the TME, which were polarized toward the M2-like phenotype. Concurrent expression of the M2-like macrophage marker CD163, along with IL-10 and PTGS2—indicators of an immunosuppressive TME—was also observed. Notably, the favorable prognostic role of MS4A1-expressing CD20-positive B cells was not apparent in tumors with high expression of IL-10 and CD163. This finding underscores the rationale for targeting M2-polarized macrophages in STS (Tsagozis et al., 2019), which can be achieved by either inhibiting M2-like TAMs or activating M1-like macrophages.

The TMEs of multiple murine sarcoma models induced tumors to produce retinoic acid (RA), which promoted the differentiation to M2-like macrophages and suppressed DCs. Reducing tumor RA production and inhibiting RA-receptor signaling enhanced anti-tumor immunity. Human sarcomas such as leiomyosarcoma, dedifferentiated liposarcoma, myxofibrosarcoma, undifferentiated pleomorphic sarcoma, and synovial sarcoma, also exhibited RA-mediated immunosuppression (Devalaraja et al., 2020). Inhibition of TREM2, an oncogenic marker found in tumor-infiltrating macrophages, increased the responsiveness to anti-PD-1 immunotherapy in sarcoma model mice. Deletion of TREM2 or administering anti-TREM2 treatments resulted in decreased tumor infiltration of $CX3CR1^+$/$MRC1^+$ TAMs and expansion of myeloid subsets that express immunostimulatory molecules improving T cell responses (Molgora et al., 2020). Moreover, various strategies investigated in other types of cancer may also hold promise for sarcoma treatment. Dual-targeted nanoparticles containing small interfering RNA against colony-stimulating factor-1 receptor depleted M2-like macrophages, increasing pro-inflammatory cytokines and decreasing immunosuppressive cytokines (Qian et al., 2017). R848, an agonist of Toll-like receptors 7 and 8, was identified as a potent driver of the M1 phenotype, and its nanoparticle-mediated delivery significantly shifted TAMs toward the M1 phenotype, leading to inhibited tumor growth (Rodell et al., 2018).

Myeloid-derived suppressor cells (MDSCs) encompass a diverse range of immature and developed myeloid cells that play crucial roles in cancer-associated immunosuppression (Nakamura and Smyth, 2020). MDSCs are divided into monocytic (M-MDSC) and granulocytic (G-MDSC) subgroups. Highfill et al. (2014) showed that G-MDSCs could suppress T cells in rhabdomyosarcoma. G-MDSCs are recruited to tumors via the CXCR2 signaling pathway, leading to immunosuppressive consequences and unfavorable outcomes for anti-PD-1 therapies in patients with rhabdomyosarcoma.

Stromal regions in the TME contain blood and lymphatic endothelial cells, MSCs, cancer-associated fibroblasts (CAFs), pericytes, and other cell types (Turley et al., 2015). CAFs are predominant in the TME, expressing α-smooth actin and fibroblast activation protein. The accumulation of CAFs in the TME is associated with a poor prognosis in many cancers; CAFs diminish anti-tumor immune responses by recruiting T_{regs} (Liu et al., 2019; Quante et al., 2011) and higher T_{reg} levels have been associated with an increased risk of local recurrence in patients with STS (Smolle et al., 2021). Various strategies have been proposed to control T_{regs} as a therapy for sarcoma. Combining imatinib with CTLA-4 blockade showed synergistic effects in gastrointestinal stromal tumor (a subtype of STS). The intracellular enzyme indoleamine 2,3-dioxygenase (IDO) participates in immune escape pathways as a major regulator of the tryptophan catabolism pathway, with kynurenine being a crucial metabolite. Kynurenine fosters immune tolerance by selectively expanding T_{regs} (Holmgaard et al., 2013). IDO1 expression was observed in 39.1% of human sarcoma cases and its blockade led to a significant decrease in both the plasmatic kynurenine-to-tryptophan ratio and tumoral kynurenine level (Nafia et al., 2020). IDO1-deficient mice showed an enhanced response to immunotherapies utilizing ICIs targeting CTLA4, PD-1, and PD-L1 (Holmgaard et al., 2013). These findings suggest IDO1 as a potential target to increase the immunogenicity of sarcomas and improve the efficacy of immunotherapies. Imatinib inhibits IDO and suppresses the number and activity of T_{regs} (Balachandran et al., 2011). The use of anti-CD25 antibodies in combination with PD-1 blockade resulted in the synergistic elimination of tumors in various cancer models, including a fibrosarcoma model (Arce Vargas et al., 2017). As the CCR4–CCL22 axis assists in the migration of T_{regs} to tumor sites, CCR4 inhibitors have been considered to inhibit T_{reg} infiltration. In a clinical study of mogamulizumab, an anti-CCR4 antibody, sarcoma patients exhibited improved prognosis when treated with durvalumab, an anti-PD-1 antibody (Zamarin et al., 2020).

MSCs, which are sources of fibroblasts and pericytes, are also well-known immune modulators that contribute to anti-tumor immunity suppression (Li et al., 2022). MSCs and CAFs have been identified in the TME of osteosarcoma (Zhou et al., 2020). Based on single-cell RNA sequencing and bioinformatic analysis, CAFs exhibit higher infiltration levels in recurrent osteosarcoma, correlating with enrichment of the EMT pathway (Huang et al., 2022). MSCs secrete factors relevant to osteosarcoma growth and immunosuppression, such as angiogenesis factors (vascular endothelial growth factor and platelet-derived growth factor) and tumor growth factor-β, which attenuate anti-tumor immune activity (Ohm et al., 2003; Zheng et al., 2018). Stromal cells have also been investigated as therapeutic targets to overcome immunosuppression. Various tumors, including osteosarcoma and undifferentiated pleomorphic sarcoma, displayed high expression of leucine-rich repeat-containing 15 (LRRC15) on CAFs and stromal cells, impeding $CD8^+$ T cell function (Krishnamurty et al., 2022; Purcell et al., 2018). A first-in-human phase I clinical trial of the antibody-drug conjugate ABBV-085, which targets LRRC15, demonstrated the ability to treat tumors by targeting stromal cells, exhibiting anti-tumor activity in patients

with osteosarcoma and undifferentiated sarcoma (Demetri et al., 2021).

SUMMARY AND PROSPECTS

Despite advancements in various chemotherapy and radiotherapy options beyond conventional surgical treatment for sarcomas, significant treatment challenges remain. The promise of immunotherapy in various solid cancers has inspired numerous studies investigating its potential in sarcomas, including cancer vaccines, immune checkpoint blockade, and ACT. However, sarcomas are often immunologically cold due to both intrinsic and extrinsic factors. Intrinsically, sarcomas may lack appropriate antigens for immune cell recognition or may induce T cell exhaustion through continuous expression of inhibitory signal. Extrinsic factors such as the TME can contribute to an immunosuppressive environment, compromising immune infiltration or activation. Despite progress in understanding the components of the immunosuppressive TMEs, many immune-resistance mechanisms remain unknown, and sarcoma TMEs can vary widely across subtypes. Therefore, a deeper understanding of TMEs in sarcomas is essential for the development of advanced immunotherapy strategies that can effectively overcome these barriers and provide more personalized and effective treatments.

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AUTHOR CONTRIBUTIONS

S.J., S.A., H.G.K., Y.-J.K., and J.-H.K. contributed to the literature search and the final manuscript. H.G.K., Y.-J.K., and J.-H.K. discussed and designed the frame of the manuscript. S.J., S.A., H.G.K., Y.-J.K., and J.-H.K. wrote the first draft of this manuscript and generated figures. S.J., S.A., D.K., J.N., J.S., J.H.K., H.J.Y., H.G.K., Y.-J.K., and J.-H.K. reviewed, revised, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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