Review Article

Immunotherapeutic strategies for sarcoma: current perspectives

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Abstract: There are more than 100 sarcoma subtypes, each of which is uncommon and challenging to diagnose. Most patients with locally advanced and unresectable sarcomas are still treated with cytotoxic chemotherapy and have low long-term survival. Therefore, novel therapeutic methods are needed to improve the prognosis of patients with sarcomas. Immunotherapy is increasingly recognized as have an essential role in the treatment of malignant tumors. Emerging strategies, such as immune checkpoint inhibitors, vaccines, and adoptive cell therapies have been investigated for the treatment of sarcomas. Advances in these immunotherapies have provided a better understanding of how immuno-oncology can be best applied to the treatment of sarcomas, including their potential as adjuvant therapies in combination strategies. In this review, we discuss the immune microenvironment and how it relates to immunoresponsiveness, focusing on the advances in immunotherapy (immune checkpoint inhibitors, vaccines and adoptive cell therapies), the use of which will hopefully lead to improved outcomes for patients with sarcomas.

Keywords: Tumor immune microenvironment, immune checkpoint inhibitor, vaccine, adoptive cell therapy, clinical significance

Introduction

Sarcomas are a group of malignant tumors originating from mesenchymal tissues that represent approximately 21% of all pediatric malignancies and approximately 1% of all adult malignancies. Despite their rarity, sarcomas are classified into more than 100 histological subtypes that are associated with the prognosis of patients. The current standard treatment protocol for sarcomas consists of surgical resection, chemotherapy, and radiation. However, these treatment options are sometimes limited for patients with metastatic and recurrent sarcomas due to organ disorders. Despite these multimodal therapies, the prognosis of patients with sarcomas has not significantly changed for decade years. Therefore, novel therapeutic methods are required to improve the outcomes of sarcoma patients [1] (Table 1).

The anti-tumor activity of the immune system was first demonstrated by William B. Coley in 1891 in a patient with an unresectable sarcoma. The sarcoma completely regressed following erysipelas, with the underlying mechanism claimed to involve the erysipelas-mediated activation of innate immunity through Toll-like receptors. In 1909, Paul Ehrlich proposed the concept of using a vaccine to allow the immune system to fight tumors. Burnet proposed the term “tumor surveillance” to describe the surveillance for tumor cells as they emerge to be recognized and destroyed by the immune system. Dunn et al. expanded the concept of “immunoediting”, which describe the balance between the immune system and malignant tumors in regard to their elimination, equilibrium, and escape. The current in-depth understanding of immunotherapy has raised hope for the use of immunotherapy in the neoadjuvant, adjuvant, and metastatic treatment of sarcomas. These works inspired many scientists to study cancer immunotherapy and to attempt to identify a cure for cancers.
Table 1. Promising targets with clinical treatment in sarcomas

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The goal of immunotherapy is to manipulate the immune system to react to malignant tumors [2]. Initial immunotherapy strategies used signaling molecules such as interleukin-2, which can activate cytotoxic T cells, to stimulate the immune system. However, this strategy was not successful. Current immunotherapy strategies enhance the immune system, such as through the use of vaccines and adoptive cell therapy, or use drugs that help inhibit the suppressive immune environment of the tumors, such as immune checkpoint inhibitors [3] (Figure 1). Increasing numbers of immunotherapies have been approved by FDA to treat malignant can-
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Figure 2. Immunotherapy used to treat sarcomas. Tumor cells are first attacked by macrophages. Then, DCs capture TAAs and present them to T cells. After activation, T cells return to tumor cells and kill them. The immunotherapy response is regulated by immune checkpoints in tumor cells. The PD-1/PD-L1 axis and the combination CTLA-4 and CD28 inhibit T cell activation. The administration of anti-PD-1, anti-PD-L1 and anti-CTLA-4 antibodies prevents immune inhibition and subsequently enhances tumor killing. In contrast to translational T cells, CAR-T recognized tumor antigens do not require MHC proteins on the tumor cell surface, which facilitates high-affinity recognition between surface antigens and natural antibodies. Abbreviations: Dendritic cells (DCs), regulatory T cells (Tregs), T cell receptor (TCR), chimeric antigen receptor T cell (CAR-T), major histocompatibility complex (MHC), programmed cell death-1 (PD-1), programmed cell death ligand-1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), tumor-associated antigen (TAA).

cers and have improved the prognosis of patients with metastatic prostate cancer [4], metastatic melanoma [5], metastatic renal cell carcinomas [6], metastatic non-small cell lung cancer [7], and Hodgkin’s lymphoma [8]. The safety and efficacies of these strategies have been investigated in clinical studies.

In this review, we will discuss the immune response to sarcomas and the immunologic markers that may predict treatment response, focusing on the current state of immunotherapy to treat sarcomas. In addition, we describe combination strategies and immune-based drug development.

Tumor immune microenvironment

The sarcoma tumor microenvironment (TME) describes a network of innate and adaptive immune cells (Figure 2). In the TME, complex interactions between tumor cells and host immune responses influence tumor evolution. Macrophages, cytotoxic T cells, and B cells can orchestrate tumor cell elimination, while populations like myeloid-derived suppressor cells, regulatory T cells (Tregs) and tumor-associated macrophages (TAMs) can suppress antitumor responses and promote malignant cell growth and tissue invasion. Thus, characterizing the tumor immune microenvironment may provide new prognostic and predictive biomarkers to enable the development of new therapeutic targets and strategies.

The immunological milieu of the tumor immune microenvironment plays an important role in the early stage of predicting responses to immunotherapy. In melanoma, a high count of
tumor-infiltrating lymphocyte (TIL) has been demonstrated to be associated with better overall survival [9]. In Ewing sarcoma, a higher number of tumor-infiltrating CD8\(^+\) T cells is associated with improved overall survival [10]. The numbers and types of TILs serve as a prognostic factor of patient survival. In addition, programmed death receptor 1 (PD-1) and its ligand (PD-L1) are correlated with TIL. PD-L1 expression has been observed in nearly 50% of dedifferentiated chondrosarcomas and was shown to be associated with a higher number of TIL [11]. In addition, PD-L1 expression on tumor cells is associated with increased number of TILs and decreased survival rate in osteosarcomas [12], whereas the presence of tumor-infiltrating Tregs is correlated with a worse prognosis. In a study of patients with metastatic Ewing sarcoma, high numbers of Tregs capable of inhibiting cytotoxic CD8\(^+\) T lymphocytes (CTLs) were observed and promoted tumor escape [10, 13].

Tumor-associated macrophages are also crucial components of this inflammatory immunological milieu. TAMs are characterized as classically (M1) and alternatively (M2) activated. M1 macrophages present antigens to T cells and highly express class II human leukocyte antigen (HLA), while M2 macrophages affect angiogenesis and tumor migration. Compared with the total number of TAMs, the balance toward M2 macrophages may confer a poorer outcome in some sarcomas, such as Ewing sarcoma [14, 15]. Studies have also shown that some sarcoma-associated factors attract and stimulate TAMs, such as colony-stimulating factor-1 (CSF-1), creating an immunosuppressive microenvironment [16, 17]. Interestingly, PD-1 and PD-L1 expression has also been correlated with TAM infiltration [18].

The immune response is a complex process, and if there is no underlying immune response, simply blocking the co-stimulatory molecules and the multiple co-inhibitory molecules (such as PD-1, PD-L1, CTLA-4) will be insufficient. If tumors do not elicit a sufficient immune response, the use of vaccines or adoptive cell strategies can enhance the immune response and lead to an antitumor response.

**Immune checkpoint inhibitors**

An encouraging approach to cancer immunotherapy is the use of checkpoint inhibitors, which involves removing the “brakes” of the immune system [19]. The 2018 Nobel Prize in Medicine was awarded to Tames Allison and Tasuku Honjo for identifying and characterizing the immunosuppressive functions of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1.

The immune checkpoint molecule CTLA-4 is a surface protein expressed on Tregs and memory T cells. CTLA-4 is upregulated when T cells are activated and competes with CD28 for binding to CD80/86 on dendritic cells (DCs). Because CTLA-4 has a stronger affinity than CD28 for CD80/86, it acts as a break and leads to T-cell anergy and apoptosis. PD-1 is another surface protein expressed on T cells that is highly expressed on chronically activated T cells. Its ligand, PD-L1, is primarily expressed on antigen-presenting cells, such as DCs, macrophages, and TILs, but it can also be expressed on tumor cells. The binding of PD-1 to its ligand PD-L1 inhibits T cells activation, proliferation, and cytotoxic secretion within tumors, leading to an attenuated antitumor immune response. Anti-CTLA-4, anti-PD-1 and anti-PD-L1 antibodies have been introduced into many tumor clinical implications, but their adoption for use in treating sarcoma has been slow [20].

D’Angelo et al. evaluated Ipilimumab (a CTLA-4 inhibitor) with or without Nivolumab (a PD-1 inhibitor) in patients with unresectable, locally advanced or metastatic sarcomas and observed that while Nivolumab alone had limited efficacy, the combination of Ipilimumab and Nivolumab demonstrated promising efficacy against some sarcomas subtypes [21]. Toulmonde et al. hypothesized that metronomic cyclophosphamide and PD-1 inhibitor have synergistic immunomodulatory effects and could benefit patients with advanced soft tissue sarcoma and gastrointestinal stromal tumors [22, 23]. Although the use of immune checkpoint inhibitors to treat sarcomas remains limited, their combined use with chemotherapy, radiation and other targeted agents have shown an improved response over checkpoint inhibitor treatment alone [24].

However, immune checkpoint inhibitors remain antigen agnostic, which presents risks with respect to immune-related adverse events such as endocrinopathies, mild-to-severe skin pathologies, hepatotoxicity, colitis, carditis, pneumonitis, renal dysfunction, and hypophysio-
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tis [25]. Thus, the use of immune checkpoint inhibitors depends on their unique toxicity but may include adjusting the duration and frequency of immunotherapy. To maximize the effectiveness of sarcoma patients, modifying the sarcoma TME and identifying new sarcoma antigens should be a priority.

Except for CTLA-4 and PD-1, many cell surface molecules can suppress tumor immunity, such as T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), indoleamine 2,3-dioxygenase (IDO), lymphocyte-activation gene 3 (LAG-3), V-domain Ig suppressor of T cell activation (VISTA) and killer cell immunoglobulin-like receptors (KIRs) [26]. These targets may be future targets in sarcoma immune treatments to further release or rev up the brakes of the immune system.

Cellular therapy

Vaccines

The vaccine was first used in osteosarcoma treatment in 1970 and was one of the first immunotherapeutic strategies used to treat cancer. The latest cancer vaccine approved by the FDA is Sipuleucel-T for metastatic prostate cancer [27].

The identification and recognition of tumor-specific or tumor-associated peptide fragments by MHC molecules to trigger the immune system is the central theme of cancer vaccines. These vaccines utilize new antigens sourced from whole tumor cells, tumor cell lysates, and tumor-related peptides. Compared to passive immunity toward cancer, active cancer vaccines induce localized inflammatory responses to cancer antigens, which can mediate antigen-specific T cell responses and long-term immunologic memory. DC vaccines are the most common vaccination approach and can be loaded with particles treated with immunoadjuvants ex vivo before being re-injected into patients. The results of a clinical vaccination trial for refractory synovial sarcoma patients with human leukocyte antigen-A24+ showed that 7 of 21 patients were stable and had a mild immune response [28]. In a placebo-controlled multicenter trial using a trivalent peptide vaccine against antigens, 136 melanoma or sarcoma patients showed no difference between the placebo and vaccine arms after metastasectomy [29]. In addition, a delayed hypersensitivity response after vaccination was associated with survival.

Viral-based vaccines are similar to peptide-based vaccines, which deliver antigens directly to DCs in vivo. Promising results have been observed for viral-based vaccines in early trials, including in a patient with metastatic recurrent synovial sarcoma after treatment with DC-targeted lentivirus LV305 [30]. Another non-cell-based approach is to directly inject tumors with attenuated oncolytic viruses that thrive in tumor cells lacking natural defenses. Similar to traditional vaccines, oncolytic viruses mediate inflammation and secondary immune responses when injected into tumor cells [31]. This approach may cause the tumor to break apart and expand the immune response by epitope spread.

Although vaccines have potential to promote anti-tumor effects by eliciting unique tumor immunological responses, studies have shown that objective response rates are low. Adjuvant agents are thought to make the immune response more robust and durable with the added benefit of reducing the number of vaccines needed and speeding up the immune response [32]. Toll-like receptor agonists, such as monophosphoryl lipid A, effectively activate DCs and CD4+ cells and have been used in several sarcoma vaccines [33]. However, further studies are needed to improve the clinical responses to vaccine therapy.

Adoptive cell therapy

Compared to immune checkpoint inhibitors and vaccines, adoptive cell therapy circumvent two steps to activate T cells and directly utilizes the activities of CTLs. To overcome tumor immunosuppressive effects, T cells from patient tumors have been used as TILs or T cells engineered to express the chimeric antigen receptors (CAR-T) and then re-injected with interleukin-2 to the same patient. The traditional adoptive cell therapy involves using naturally occurring tumor-reactive lymphocytes. With the development of genetically engineered lymphocytes, CAR-T has further enhanced the successful application of adoptive cell therapy in cancer management [34].

To overcome the mechanisms leading to tumor immune escape, CAR-T has been developed with a genetically modified T cell receptor spe-
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cific to tumor-associated antigens [35]. During an endogenous immune response, CTLs detect tumor cells by recognizing self-antigens, which are presented on MHC class I molecules to induce a CTL response, while MHC class II antigen presentation drives CD4+ helper T cell response [36]. Compared to a natural TCR, CAR-T is not restricted by HLA. CAR receptors are engineered using the extracellular binding domain of antibodies fused to the intracellular signaling domain of T-cell receptors. This approach facilitates a high-affinity recognition between surface antigens and natural antibodies, which are best exemplified in hematological malignancies by CD19 targeted therapy [37, 38]. In a study of CAR-T therapy, targeting human epidermal growth factor receptor 2 in patients with osteosarcoma and Ewing sarcoma resulted in tumor necrosis [39].

For CAR-T therapy, two primary criteria need to be fulfilled to make it efficacious. First, CAR-T cells should target epitopes selectively expressed on the sarcomas to prevent toxicity toward in normal tissues. Second, the target should be widely expressed on sarcoma metastases. As GD2, NY-ESO-1, and MAGE are specifically expressed in many sarcomas, they may be useful to study the specific effects of CAR-T cells on these antigens. Thus, specific cataloging of neoantigens associated with sarcomas will be needed to develop CAR-related strategies.

Adverse events related to adoptive cell therapy are described as cytokine release syndrome, which is a systemic inflammatory response related to the activation of CAR-T cells. Cytokine release syndrome ranges from mild to severe symptoms, including fever, fatigue, hypotension, respiratory failure, and multi-organ failure. The efficacy and safety of CAR-T therapy need further investigation.

Conclusion and further directions

The field of immunotherapy is rapidly evolving, and promising strategies such as immune checkpoint inhibitors, vaccines and adoptive cell therapy may be synergistic. The complexity of the immune system and the disappointing activity of the monotherapies to date suggests that a combined strategy will be important to optimize immunotherapies to treat. According to the results of a phase 1 trial for the CMB305 vaccine and observations that the extreme response to the vaccine resulted in an increased proportion of NY-ESO-1 positive and PD-1 positive T cells, the CMB305 protocol has been used in combination with atezolizumab in a phase 2 randomized study of 88 patients with locally advanced or metastatic NY-ESO-1 positive sarcomas [40, 41]. Interim results of 36 patients followed for 7 months show an improved median PFS for the combination arm of 2.6 months over 1.4 months for atezolizumab alone [41]. Thus, the combined use of vaccines and immune checkpoint inhibitors may be an effective treatment approach. Checkpoint inhibitors may also enhance CAR-T therapy, which may be limited by immunosuppressive cytokines, increased Tregs, an absence of target antigen expression, anti-antibody-induced T-cell exhaustion, and the upregulation of PD-1 [42]. In addition, based on encouraging preclinical data, checkpoint inhibitors have also been used together with vaccines and CAR-T therapy in phase I trials [43, 44].

Drug resistance of sarcomas frequently occurs during treatment, especially in metastatic sarcomas. Thus, understanding the changes that occur in drug resistant tumors should be performed to elucidate the associated mechanisms. Previous studies have shown that the drug-resistant of tumors is associated with cancer stem cells [45, 46], and an accumulation of these resistant cells in the tumor cell population may be due to epigenetic reversal of the differentiation status [47]. Therefore, understanding the mechanisms of drug resistance in genetically simple sarcomas is beneficial from the standpoint of identifying the accompanying driver gene mutations as well as understanding the perspective of epigenetics that change the fate of cells from differentiation to more pluripotent state.

In summary, immunotherapy should be considered as a candidate for standard treatment in combination with chemotherapy, radiation therapy and surgery. Although the clinical prospects of locally advanced and metastatic sarcomas are limited, with the innovation of genetic profiling and cytogenetics, there has been an increased appreciation for the complexity and heterogeneity of sarcomas that has led to the development to more fundamental treatments base on biology than on histological appearance. Rather than committing a one-size-fits-all treatment method for patients, precision medicine is becoming mainstream in modern cancer
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therapy. Unprecedented advances in the acquisition of genomic information and the availability of targeted therapies have provided a new paradigm for investigating sarcoma treatments. Furthermore, as seen with traditional chemotherapeutic drugs, tumors utilize multiple approaches to resist immunotherapy, indicating the need for a combination approach to achieve a meaningful and durable response. Challenges remain in developing immunotherapy for sarcomas. A great deal of work remains to be performed, and immunotherapy holds the promise of a breakthrough that will revolutionize the treatment of sarcomas.

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Disclosure of conflict of interest

None.

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