Am J Transl Res 2020;12(12):7693-7701
www.ajtr.org /ISSN:1943-8141/AJTR0114249

Review Article

Immunotherapeutic strategies for sarcoma: current perspectives

Xueyao Li[1,2*], Gangyang Wang[1*], Zhengdong Cai[1], Wei Sun[1]

[1]Department of Orthopaedics, Shanghai Bone Tumor Institute, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China; [2]Puyang People's Hospital, Xinxiang Medical University, Xinxiang 453000, Henan, China. [*]Equal contributors.

Received May 12, 2020; Accepted October 24, 2020; Epub December 15, 2020; Published December 30, 2020

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

Approach	Strategy	Clinical pharmacy	Citations
Immune checkpoint inhibitor	Anti-CTLA-4	Tremelimumab, Ipilimumab	[48, 49]
	Anti-PD-1	Pembrolizumab, Nivolumab	[48]
	Anti-PD-L1	Avelumab, Atezolizumab, Durvalumab	[48]
Vaccines	DC-based vaccine	Autologous tumor cells	[50]
		Lysate-based	[51]
		Peptide-based	[4, 52]
	Non-cell-based vaccine	Viral-based vaccine	[30]
		Oncolytic viruses	[31]
Adoptive cell therapy	Tumor antigens	CAR-T	[53]
		TCR-T	[54]

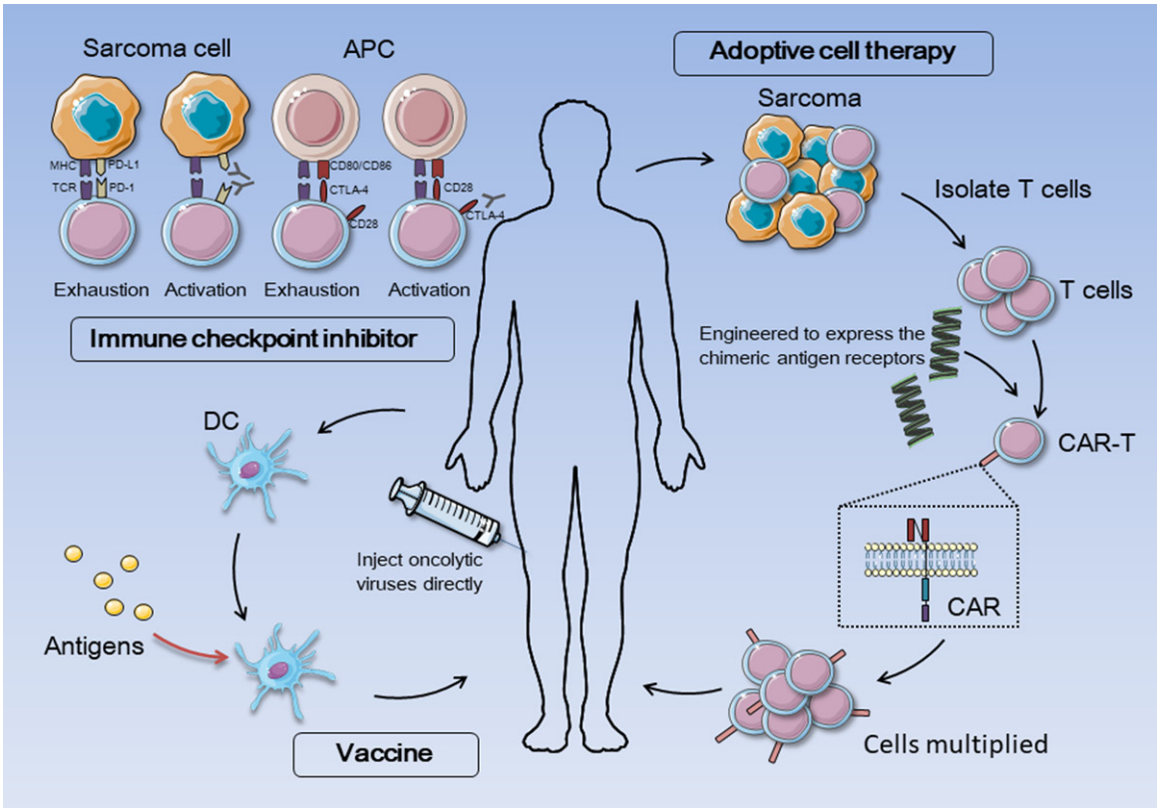

Figure 1. Summarization of the current mainstream immunotherapy approaches. Immune checkpoint inhibitor treatments are designed to reinvigorate the suppressed or suboptimal immune response. Vaccines induce tumor immune responses through antigen presentation and priming new T cell responses. Compared to immune checkpoint inhibitor and vaccine approaches, adoptive cell therapy directly utilizes the management of $CD8^+$ cytotoxic T lymphocytes (CTLs). Abbreviations: Antigen-presenting cell (APC), Dendritic cell (DC), chimeric antigen receptor T cell (CAR-T), programmed cell death-1 (PD-1), programmed cell death ligand-1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).

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 active 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-

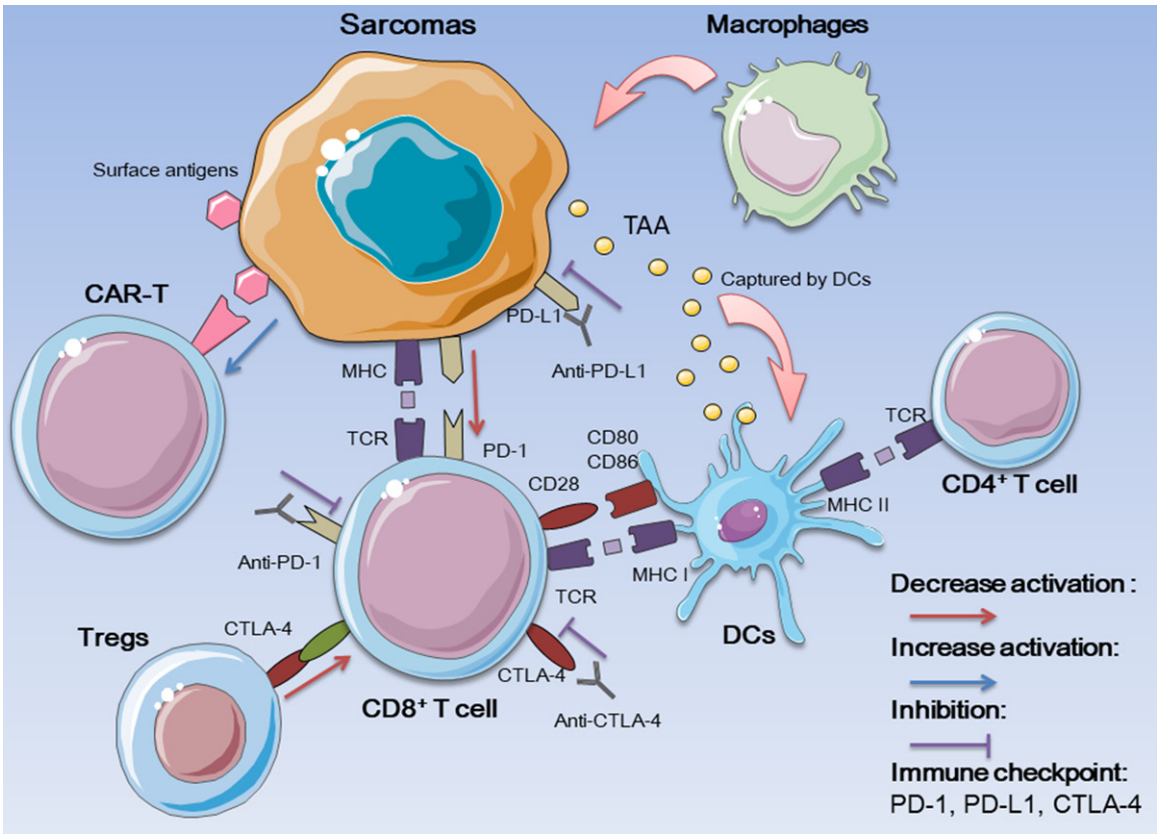

Figure 2. Immunotherapy used to treat sarcomas. Tumor cells are first attacked by macrophages. Then, DCs capture TAAs and present them to T cells. After activation, T cells return to tumor cells and kill them. The immunotherapy response is regulated by immune checkpoints in tumor cells. The PD-1/PD-L1 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 facilities 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 hypophysi-

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-

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 facilities 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 describe 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

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.

Acknowledgements

This work was supported by Shanghai Sailing Program (No. 19YF1440200) and Shanghai Jiaotong University Translation Medicine Cross Research Fund (ZH2018QNA17) Doctoral Innovation Fund of Shanghai Jiaotong University School of Medicine (No. BXJ201732).

Disclosure of conflict of interest

None.

Address correspondence to: Wei Sun, Department of Orthopaedics, Shanghai Bone Tumor Institute, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 20000, China. E-mail: viv-sun@163.com

References

[1] Sebio A, Wilky B, Keedy V and Jones RJ. The current landscape of early drug development for patients with sarcoma in the immunotherapy era. Future Oncol 2018; 14: 1197-1211.

[2] Pham T, Roth S, Kong J, Guerra G, Narasimhan V, Pereira L, Desai J, Heriot A and Ramsay R. An update on immunotherapy for solid tumors: a review. Ann Surg Oncol 2018; 25: 3404-3412.

[3] Hoffman RM, Singh AS and Eilber FC. Potential of immunotherapy for sarcoma. Cancer 2017; 123: 1488-1489.

[4] Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW and Schellhammer PF; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010; 363: 411-422.

[5] Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, Delman KA, Spitler LE, Puzanov I, Agarwala SS, Milhem M, Cranmer L, Curti B, Lewis K, Ross M, Guthrie T, Linette GP, Daniels GA, Harrington K, Middleton MR, Miller WH Jr, Zager JS, Ye Y, Yao B, Li A, Doleman S, VanderWalde A, Gansert J and Coffin RS. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol 2015; 33: 2780-2788.

[6] Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, Vaishampayan UN, Drabkin HA, George S, Logan TF, Margolin KA, Plimack ER, Lambert AM, Waxman IM and Hammers HJ. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol 2015; 33: 1430-1437.

[7] Kazandjian D, Suzman DL, Blumenthal G, Mushti S, He K, Libeg M, Keegan P and Pazdur R. FDA approval summary: nivolumab for the treatment of metastatic non-small cell lung cancer with progression on or after platinum-based chemotherapy. Oncologist 2016; 21: 634-642.

[8] Chen R, Zinzani PL, Fanale MA, Armand P, Johnson NA, Brice P, Radford J, Ribrag V, Molin D, Vassilakopoulos TP, Tomita A, von Tresckow B, Shipp MA, Zhang Y, Ricart AD, Balakumaran A and Moskowitz CH; KEYNOTE-087. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic hodgkin lymphoma. J Clin Oncol 2017; 35: 2125-2132.

[9] Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P and Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 1996; 77: 1303-1310.

[10] Berghuis D, Santos SJ, Baelde HJ, Taminiau AH, Egeler RM, Schilham MW, Hogendoorn PC and Lankester AC. Pro-inflammatory chemokine-chemokine receptor interactions within the Ewing sarcoma microenvironment determine CD8(+) T-lymphocyte infiltration and affect tumour progression. J Pathol 2011; 223: 347-357.

[11] Kostine M, Cleven AH, de Miranda NF, Italiano A, Cleton-Jansen AM and Bovée JV. Analysis of PD-L1, T-cell infiltrate and HLA expression in chondrosarcoma indicates potential for response to immunotherapy specifically in the dedifferentiated subtype. Mod Pathol 2016; 29: 1028-1037.

[12] Koirala P, Roth ME, Gill J, Piperdi S, Chinai JM, Geller DS, Hoang BH, Park A, Fremed MA, Zang X and Gorlick R. Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma. Sci Rep 2016; 6: 30093.

[13] Brinkrolf P, Landmeier S, Altvater B, Chen C, Pscherer S, Rosemann A, Ranft A, Dirksen U, Juergens H and Rossig C. A high proportion of bone marrow T cells with regulatory phenotype (CD4+CD25hiFoxP3+) in Ewing sarcoma patients is associated with metastatic disease. Int J Cancer 2009; 125: 879-886.

[14] Teicher BA, Bagley RG, Rouleau C, Kruger A, Ren Y and Kurtzberg L. Characteristics of human Ewing/PNET sarcoma models. Ann Saudi Med 2011; 31: 174-182.
[15] Saha D, Martuza RL and Rabkin SD. Macrophage polarization contributes to glioblastoma eradication by combination immunovirotherapy and immune checkpoint blockade. Cancer Cell 2017; 32: 253-267, e5.
[16] West RB, Rubin BP, Miller MA, Subramanian S, Kaygusuz G, Montgomery K, Zhu S, Marinelli RJ, De Luca A, Downs-Kelly E, Goldblum JR, Corless CL, Brown PO, Gilks CB, Nielsen TO, Huntsman D and van de Rijn M. A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. Proc Natl Acad Sci U S A 2006; 103: 690-695.
[17] Ganjoo KN, Witten D, Patel M, Espinosa I, La T, Tibshirani R, van de Rijn M, Jacobs C and West RB. The prognostic value of tumor-associated macrophages in leiomyosarcoma: a single institution study. Am J Clin Oncol 2011; 34: 82-86.
[18] Feng Y, Shen J, Gao Y, Liao Y, Cote G, Choy E, Chebib I, Mankin H, Hornicek F and Duan Z. Expression of programmed cell death ligand 1 (PD-L1) and prevalence of tumor-infiltrating lymphocytes (TILs) in chordoma. Oncotarget 2015; 6: 11139-11149.
[19] Wisdom AJ, Mowery YM, Riedel RF and Kirsch DG. Rationale and emerging strategies for immune checkpoint blockade in soft tissue sarcoma. Cancer 2018; 124: 3819-3829.
[20] Veenstra R, Kostine M, Cleton-Jansen AM, de Miranda NF and Bovée JV. Immune checkpoint inhibitors in sarcomas: in quest of predictive biomarkers. Lab Invest 2018; 98: 41-50.
[21] D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, Jahagirdar BN, Antonescu CR, Horvath E, Tap WD, Schwartz GK and Streicher H. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol 2018; 19: 416-426.
[22] Toulmonde M, Penel N, Adam J, Chevreau C, Blay JY, Le Cesne A, Bompas E, Piperno-Neumann S, Cousin S, Grellety T, Ryckewaert T, Bessede A, Ghiringhelli F, Pulido M and Italiano A. Use of PD-1 targeting, macrophage infiltration, and IDO pathway activation in sarcomas: a phase 2 clinical trial. JAMA Oncol 2018; 4: 93-97.
[23] Mkrtichyan M, Najjar YG, Raulfs EC, Abdalla MY, Samara R, Rotem-Yehudar R, Cook L and Khleif SN. Anti-PD-1 synergizes with cyclophosphamide to induce potent anti-tumor vaccine effects through novel mechanisms. Eur J Immunol 2011; 41: 2977-2986.
[24] Zuo W and Zhao L. Recent advances and application of PD-1 blockade in sarcoma. Onco Targets Ther 2019; 12: 6887-6896.
[25] Dyson KA, Stover BD, Grippin A, Mendez-Gomez HR, Lagmay J, Mitchell DA and Sayour EJ. Emerging trends in immunotherapy for pediatric sarcomas. J Hematol Oncol 2019; 12: 78.
[26] Dempke WCM, Fenchel K, Uciechowski P and Dale SP. Second- and third-generation drugs for immuno-oncology treatment-The more the better? Eur J Cancer 2017; 74: 55-72.
[27] Tsiatas M, Mountzios G and Curigliano G. Future perspectives in cancer immunotherapy. Ann Transl Med 2016; 4: 273.
[28] Kawaguchi S, Tsukahara T, Ida K, Kimura S, Murase M, Kano M, Emori M, Nagoya S, Kaya M, Torigoe T, Ueda E, Takahashi A, Ishii T, Tatezaki S, Toguchida J, Tsuchiya H, Osanai T, Sugita T, Sugiura H, Ieguchi M, Ihara K, Hamada K, Kakizaki H, Morii T, Yasuda T, Tanizawa T, Ogose A, Yabe H, Yamashita T, Sato N and Wada T. SYT-SSX breakpoint peptide vaccines in patients with synovial sarcoma: a study from the Japanese Musculoskeletal Oncology Group. Cancer Sci 2012; 103: 1625-1630.
[29] Chapman PB, Morrisey D, Panageas KS, Williams L, Lewis JJ, Israel RJ, Hamilton WB and Livingston PO. Vaccination with a bivalent G(M2) and G(D2) ganglioside conjugate vaccine: a trial comparing doses of G(D2)-keyhole limpet hemocyanin. Clin Cancer Res 2000; 6: 4658-4662.
[30] Pollack SM, Lu H, Gnjatic S, Somaiah N, O'Malley RB, Jones RL, Hsu FJ and Ter Meulen J. First-in-human treatment with a dendritic cell-targeting lentiviral vector-expressing NY-ESO-1, LV305, induces deep, durable response in refractory metastatic synovial sarcoma patient. J Immunother 2017; 40: 302-306.
[31] Pollack S, Ingham M, Spraker M and Schwartz GK. Emerging targeted and immune-based therapies in Sarcoma. J Clin Oncol 2018; 36: 125-135.
[32] Linch M, Miah AB, Thway K, Judson IR and Benson C. Systemic treatment of soft-tissue sarcoma-gold standard and novel therapies. Nat Rev Clin Oncol 2014; 11: 187-202.
[33] Coler RN, Bertholet S, Moutaftsi M, Guderian JA, Windish HP, Baldwin SL, Laughlin EM, Duthie MS, Fox CB, Carter D, Friede M, Vedvick TS and Reed SG. Development and characterization of synthetic glucopyranosyl lipid adjuvant system as a vaccine adjuvant. PLoS One 2011; 6: e16333.
[34] Curran K and Brentjens RJ. Chimeric antigen receptor T cells for cancer immunotherapy. J Clin Oncol 2015; 33: 1703-1706.
[35] Thanindratarn P, Dean DC, Nelson SD, Hornicek FJ and Duan Z. Chimeric antigen receptor T (CAR-T) cell immunotherapy for sarcomas:

from mechanisms to potential clinical applications. Cancer Treat Rev 2020; 82: 101934.
[36] Miwa S, Nishida H and Tsuchiya H. Current status of immunotherapy for sarcomas. Immunotherapy 2017; 9: 1331-1338.
[37] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS and Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015; 385: 517-528.
[38] Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL and Grupp SA. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371: 1507-1517.
[39] Ahmed N, Brawley V, Hegde M, Robertson C, Ghazi A, Gerken C, Liu E, Dakhova O, Ashoori A, Corder A, Gray T, Wu M, Liu H, Hicks J, Rainusso N, Dotti G, Mei Z, Grilley B, Gee A, Rooney C, Brenner M, Heslop H, Wels W, Wang L, Anderson P and Gottschalk S. Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 2015; 33: 1688-1696.
[40] Somaiah N, Chawla SP, Block MS, Morris JC, Do KT, Kim JW, Druta M, Sankhala KK, Hwu P, Gnjatic S, Lu H, Kenney RT, Bohac GC and Pollack S. Immune response, safety, and survival impact from CMB305 in NY-ESO-1+ recurrent soft tissue sarcomas (STS). 2017; 35: 11006-11006.
[41] Pollack SM. The potential of the CMB305 vaccine regimen to target NY-ESO-1 and improve outcomes for synovial sarcoma and myxoid/round cell liposarcoma patients. Expert Rev Vaccines 2018; 17: 107-114.
[42] Sun C, Dotti G and Savoldo B. Utilizing cell-based therapeutics to overcome immune evasion in hematologic malignancies. Blood 2016; 127: 3350-3359.
[43] Fu J, Malm IJ, Kadayakkara DK, Levitsky H, Pardoll D and Kim YJ. Preclinical evidence that PD1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. Cancer Res 2014; 74: 4042-4052.
[44] Sierro SR, Donda A, Perret R, Guillaume P, Yagita H, Levy F and Romero P. Combination of lentivector immunization and low-dose chemotherapy or PD-1/PD-L1 blocking primes self-reactive T cells and induces anti-tumor immunity. Eur J Immunol 2011; 41: 2217-2228.
[45] Eyler C and Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. J Clin Oncol 2008; 26: 2839-2845.
[46] Potter JW, Jones KB and Barrott JJ. Sarcoma-The standard-bearer in cancer discovery. Crit Rev Oncol Hematol 2018; 126: 1-5.
[47] Easwaran H, Tsai HC and Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell 2014; 54: 716-727.
[48] Sengupta R and Honey K. AACR cancer progress report 2018: harnessing research discoveries for patient benefit. Clin Cancer Res 2018; 24: 4351.
[49] Nathenson MJ, Conley AP and Sausville E. Immunotherapy: a new (and old) approach to treatment of soft tissue and bone sarcomas. Oncologist 2018; 23: 71-83.
[50] Goldberg JM, Fisher DE, Demetri GD, Neuberg D, Allsop SA, Fonseca C, Nakazaki Y, Nemer D, Raut CP, George S, Morgan JA, Wagner AJ, Freeman GJ, Ritz J, Lezcano C, Mihm M, Canning C, Hodi FS and Dranoff G. Biologic activity of autologous, granulocyte-macrophage colony-stimulating factor secreting alveolar soft-part sarcoma and clear cell sarcoma vaccines. Clin Cancer Res 2015; 21: 3178-3186.
[51] Merchant MS, Bernstein D, Amoako M, Baird K, Fleisher TA, Morre M, Steinberg SM, Sabatino M, Stroncek DF, Venkatasan AM, Wood BJ, Wright M, Zhang H and Mackall CL. Adjuvant immunotherapy to improve outcome in high-risk pediatric sarcomas. Clin Cancer Res 2016; 22: 3182-3191.
[52] Krishnadas DK, Shusterman S, Bai F, Diller L, Sullivan JE, Cheerva AC, George RE and Lucas KG. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. Cancer Immunol Immunother 2015; 64: 1251-1260.
[53] Rosenberg SA and Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 2015; 348: 62-68.
[54] Robbins P, Kassim S, Tran T, Crystal J, Morgan R, Feldman S, Yang J, Dudley M, Wunderlich J, Sherry R, Kammula U, Hughes M, Restifo N, Raffeld M, Lee C, Li Y, El-Gamil M and Rosenberg SA. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res 2015; 21: 1019-1027.