Current status of immunotherapy for sarcomas

Special report (1500-3000 words)

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ABSTRACT

Although the development of anticancer drugs has improved the outcomes of bone and soft tissue sarcomas, the clinical outcome of patients with relapsed sarcomas remains unsatisfactory due to therapeutic toxicities and resistance to anticancer drugs. Therefore, novel therapeutic modalities are needed to improve the outcome of patients with bone and soft tissue sarcomas. Dendritic cells present tumor antigens and stimulate immune responses, and immune cells, such as cytotoxic T lymphocytes, kill tumor cells by recognizing tumor antigens. However, immune-suppressive conditions by immune regulator PD-1, CTLA-4, and regulatory T cells help tumor growth and progression. In this report, current immunotherapies including cellular immunotherapy and checkpoint inhibitors are introduced, and the advantages and disadvantages of the treatments are discussed.

Current treatment of sarcomas

Bone and soft tissue sarcomas are rare, heterogeneous solid tumors of mesenchymal origin. Current standard treatment options for bone and soft tissue sarcomas consist of surgical resection, chemotherapy, and radiation therapy. Before the introduction of chemotherapy, long-term survival occurred in only 20 to 40% of patients with bone sarcomas and in only 35% of patients with soft tissue sarcomas [1]. Since the 1970s, chemotherapy has significantly improved the outcomes of sarcomas, and a majority of the patients without metastasis at the time of initial diagnosis have long survival [1]. Five-year survival of 60 to 80% has been reported for patients receiving chemotherapy with surgical resection [2–6]. Despite intensive treatment, including chemotherapy, surgery, and radiation therapy, 6–15% of patients develop recurrent disease [7–10], and the outcome of the patients with recurrent or metastatic sarcomas remains poor [11]. Current treatment options are sometimes quite limited for patients with recurrent or metastatic sarcomas because of resistance to chemotherapy and organ disorders, such as kidney and heart disorders, caused by repeated chemotherapy. Although metastasectomy can be performed in patients with a small number of metastatic lesions, only a part of these patients has long term survival [12]. Although the efficacies of new anticancer agents for sarcomas were investigated in clinical trials [13–16], no remarkable data can currently be derived [17]. Therefore, novel therapeutic modalities are required for improving outcomes in patients with advanced sarcomas. Recent elucidation of the relationships between cancer cells and immune systems has contributed to the development of immunotherapy. Effects and limitations for immunotherapies for sarcomas are discussed in this report.

Target of cellular immunotherapy

Immune responses are classified into innate and adaptive immunity [18]. Innate immunity, comprising neutrophils, macrophages, and natural killer cells, provide initial defense against invading microbes. Cytokines mediate various activities of the cells involved in innate immunity. T and B

lymphocytes, key components of adaptive immunity following initial innate immunity, play central role in eliminating infectious pathogens, virus-infected cells, and cancer cells, and in generating antigen-specific memory cells. Adaptive immunity consists of humoral- and cell-mediated immunity. T lymphocytes recognize antigens presented by major histocompatibility complexes (MHCs) on the surface of dendritic cells (DCs). CD4+ and CD8+ T cells recognize antigens in the context of the MHC. Primed and activated T cells differentiate into mature effector cells, recognize virus infected cells and cancer cells, and eliminate them from the body.

An effective immune system identifies cancer cells via detection of tumor-associated antigens (TAAs). TAAs are presented on the surface of the cell by human leukocyte antigen (HLA) class I and is targeted by cytotoxic T lymphocytes. Recent studies of TAAs have resulted in the identification of TAAs expressed by various cancer types including breast, lung, colon, liver, and lung. Cytotoxic T lymphocytes (CTL) are immune cells responsible for killing tumor cells. CTLs detect tumor cells by recognizing peptide fragments of endogenous proteins, which are presented to CTLs in complex with MHC class I molecules on the surface of the tumor cells. Antigen-presenting cells (APCs) induce the CTL response by delivering costimulatory signals between APCs and CTLs to CD4+ and CD8+ cells. On the other hand, DCs, one of the potent APCs, have the ability to take up apoptotic cells, process them, and present the tumor antigens. DCs play a pivotal role in the decision to promote or inhibit immune responses, including CTL responses [19]. In normal conditions, cancer cells with abnormal antigens are eliminated by the immune responses. On the other hand, insufficient function of the immune system exists since the early stages of cancer, and some cancer cells escape from the immune system. Furthermore, cancer cells suppress immune checkpoints and the immune system by recruitment of cells with immune-suppressive functions, such as regulatory T cells and myeloid-derived suppressor cells.

Various subtypes of sarcomas express TAAs that allow the development of cellular immunotherapies [20]. In particular, some sarcomas have specific chromosomal translocations that may

serve as unique targets for immunotherapy [21]. Synovial sarcoma and myxoid/round cell liposarcoma are translation-driven malignancies and often express high levels of self-antigen, notably NY-ESO-1, a member of the MAGE and GAGE family, or developmental antigens such as the fetal acetylcholine receptor [22–26]. DC-based vaccines target TAAs including fusion proteins, such as EWS-FLI-1 expressed in EWS or SYT-SSX2 expressed in synovial sarcoma [27,28]. These fusion proteins have been thought to be promising candidates as targets for immunotherapy. Although some TAAs have been identified in some sarcomas, a number of unknown TAAs may exist in sarcoma cells. In immunotherapy utilizing DCs, tumor lysate (TL) is commonly used as the pool of TAAs to sensitize immune cells, including DCs.

Recent advancements in cancer immunity revealed the presence of immunity-inhibition in cancer patients [29]. To prevent an excessive immune response, inhibition or elimination of activated T lymphocyte is regulated by immune checkpoints. Activated T lymphocytes, particularly cytotoxic T lymphocytes, express immune checkpoint receptor-programmed cell death protein 1 (PD-1) [30], and inhibit their survival by signals from PD-ligand 1 (PD-L1) and PD-ligand 2 (PD-L2). The expression of PD-L1 is seen in DCs and non-immune cells, such as cancer cells, whereas the expression of PD-L2 was seen only in DCs, macrophages, and B lymphocytes [31]. In particular, cancer cells highly expressing PD-L1 and the interaction of PD-1 and PD-L1 downregulate the function of T cells expressing PD-1 within the cancer microenvironment [32,33]. CTLA-4 is a regulatory molecule, involved in the downregulation of T cells and cytokine production [34]. Co-stimulatory signals by B7/CD28 binding result in T cell activation and chemokine production. Effector T cells regulate expression of CTLA-4 after induction of the costimulatory interaction between CD28 and B7 on APCs. However, regulatory T cells highly express CTLA-4 constitutively to suppress the activation of cytotoxic lymphocytes. The predominance of co-inhibitory pathways enables tumor cells to evade immune system control [35]. Self-tolerance and immunologic homeostasis are regulated by B7/CTLA-4 binding. Based on basic

research, anti-CTLA-4 and anti-PD-1 antibodies have been introduced for clinical use for several types of cancer [36].

Therapeutic vaccine in sarcomas

The first report of cancer immunotherapy was performed by William B. Coley [37]. He observed that a patient with an inoperable sarcoma that suffered a *Streptococcus pyogenes* infection twice showed complete remission. Base on this case, Coley treated approximately 1000 patients with inoperable malignant tumors with live or inactivated bacteria, known as "Coley's toxins", achieving complete remission in 10% of the patients. In the 1970s, Marcove reported that immunization using autologous tumor lysate significantly improved overall survival and that 9 of 21 patients had long term survival [38]. In a clinical trial of tumor-specific peptide vaccination combined with IL-2 for patients with Ewing sarcoma and alveolar rhabdomyosarcoma, no clinical benefit and immunological response were observed [39]. A cocktail peptide vaccination phase II study for 20 patients with bone and soft tissue sarcoma showed that stable disease was observed in 6 of 20 patients, and that there was regression of pulmonary metastasis and long-survival in patients with malignant fibrous histiocytoma and synovial sarcoma, respectively [40]. A clinical vaccination trial for HLA-A24+ patients with refractory synovial sarcoma reported by Kawaguchi showed that 7 of 21 patients showed stable disease with minor immunological responses [41]. Although vaccination with antigenic peptides has some efficacy and potential to elicits T cell responses directed at vaccine peptides, the reported objective response rates are low. It is thought that immunological adjuvant agents are needed to improve the clinical response to vaccine therapy,

Cytokine therapy for sarcomas

Immune system is regulated by cytokines, and cytokines such as interleukin-2 (IL-2) and interferons (IFNs) have been used for immunotherapy [42–44]. Ito reported that 2 of 3 patients with

metastatic osteosarcoma treated with human leukocyte interferon showed remission of the metastatic tumors [42]. Schwinger reported that high-dose IL-2 treatment in 4 patients with metastatic osteosarcomas and 2 patients with metastatic Ewing sarcoma showed 2 patients with osteosarcoma obtained complete remission [43]. However, all the patients showed adverse events such as fatigue, anorexia, diarrhea, nausea, vomiting, and fever, and the treatment had to be stopped in 2 patients. EURAMOS-1 study, a recent study in Europe, investigated the efficacy of the use of adjuvant chemotherapy with pegylated interferon alfa-2b (IFN-α-2b) in patients with osteosarcoma, and no significant improvement of the event-free survival by IFN-α-2b was observed in the study patients [44]. These studies indicate that cytokine therapies may improve the prognosis of bone and soft tissue sarcoma to some extent.

Cellular immunotherapy for sarcomas

Although immunotherapy as a novel modality for cancer treatment has been markedly developed, only a small number of clinical trials on cellular immunotherapy in patients with sarcoma have been reported (Table 1).

In a phase 1 study of DC immunotherapy using DCs pulsed with TL, keyhole limpet hemocyanin (KLH), lipopolysaccharide, and interferon gamma (IFN-γ), immune responses were induced in 3 of the 6 patients and no serious adverse events were observed in the study patients [45]. Himoudi reported that a phase 1 trial of immunotherapy using DCs pulsed with TL and KLH showed that significant anti-tumor responses were induced in only 2 out of 12 patients with no evidence of clinical benefit [46]. Geiger reported that a phase 1 trial of TL-pulsed DCs in pediatric solid tumors showed significant regression of metastatic sites in 1 patient and stable disease in 5 patients out of 10 patients [47]. Suminoe reported DC-based immunotherapy using tumor-specific synthetic peptide or TL in 5 patients with refractory solid tumors (Ewing sarcoma, synovial sarcoma, and neuroblastoma) [48]. In the report,

one patient with Ewing sarcoma had a residual tumor disappear following DC therapy, whereas 4 patients had progression of the disease. Merchant reported a clinical trial of adjuvant immunotherapy using autologous lymphocytes and TL-pulsed DCs following standard antineoplastic treatment [49]. In the study, the five-year overall survival rate in patients with or without immunotherapy were 62% and 29%, respectively, although there was no statistical significance by multivariate analysis. We previously reported a phase 1/2 study of DC-based immunotherapy in 37 patients with refractory bone and soft tissue sarcoma [50]. In the study, DCs were treated with or without TL, TNF-α, or OK-432, a penicillin-inactivated and lyophilized preparation of *Streptococcus pyogenes* [51]. The study patients showed significant elevation of serum IFN-γ and serum IL-12 after the DC-based immunotherapy, and clinical responses (partial response and stable disease) were observed in 7 patients (20%) after the DC-based immunotherapy (stable disease in 6 patients and tumor regression in 1 patient), and no patients had severe adverse events [50]. In the previous studies of DC-based immunotherapy, no significant toxicity associated with the immunotherapy was reported, whereas some patients experienced induration at the injection site and fever. DC therapy may represent a feasible, generally well-tolerated therapy in patients with refractory sarcoma, owing to its safety and immunological responses. Although DC-based immunotherapy was effective only in a small part of the patients with advanced sarcoma, it can be a promising treatment for prevention of the relapse of sarcomas in patients.

Recently, adoptive T cell transfer therapy showed a high response rate in patients with synovial sarcoma. Cancer germline antigen NY-ESO-1 is expressed in 10% to 50% of metastatic melanomas, lung, breast, prostate, thyroid, and ovarian cancers as well as in 70% to 80% of synovial cell sarcomas [22,25,26]. Robbins reported a clinical trial utilizing the adoptive transfer of autologous peripheral blood mononuclear cells (PBMC) that were retrovirally transduced with a high-affinity T cell receptor directed against NY-ESO-1. The study showed objective clinical responses (complete response and partial response) in 11 of 17 patients with synovial sarcoma [25]. In the study, no toxicity associated with the

transferred cells was observed, although all patients experienced transient neutropenia and thrombocytopenia associated with the adjuvant use of IL-2. Phase 1/2 clinical study of T cells expressing an HER2-specific chimeric antigen receptor in patients with HER2-positive sarcomas [52] showed that 4 of 17 patients had stable disease for 12 weeks to 14 months without significant toxicity. Although immunotherapies using TAA-specific T cells resulted in a significant result for synovial sarcoma, the treatment can be used in only some bone and soft tissue sarcomas because TAA remains unknown in most type of sarcomas.

Immune checkpoint inhibitors

The expression of PD-1 and PD-L1 reflects antitumor immunity and associated with prognosis of the patients with various cancers. Nowicki reported that PD-1 and PD-L1 expressions was significantly higher in metastatic tumors than in primary tumors and that PD-1 positivity was significantly associated with progression-free survival in patients with synovial sarcoma [53]. Zheng reported that PD-1 was significantly upregulated in both peripheral CD4+ and CD8+ T cells from patients with osteosarcoma and that patients with metastasis showed significantly higher levels of PD-1 in CD4+ T cells than in those without metastasis [54]. High expression of PD-1 and PD-L1 were reported in pleomorphic sarcomas (50%), malignant peripheral nerve sheath tumors (50%), and Kaposi sarcomas (80%), whereas synovial sarcomas had only a low expression of PD-1 and PD-L1 (25%) [55]. Kim reported that high expression of PD-1 and PD-L1 was observed in undifferentiated sarcoma, angiosarcoma, epithelioid sarcoma, rhabdomyosarcoma, whereas only a low expression of PD-1 and PD-L1 was observed in myxoid liposarcoma, well-differentiated liposarcoma, and myxofibrosarcoma [56]. In the report, PD-1 and PD-L1 expression were associated with clinical stage, distant metastasis, histological grade, poor differentiation of tumor, and tumor necrosis in patients with sarcomas, and PD-1 and PD-L1 expression significantly correlated with overall survival and event-free survival in patients with sarcoma. Pollack investigated the

expression of PD-1 and PD-L1 and tumor subtypes and reported that a high-grade tumor was associated with higher expression of PD-1 and PD-L1 [57]. In the report, high expression of PD-1 and PD-L1 was observed in undifferentiated pleomorphic sarcoma and leiomyosarcoma, whereas very few synovial sarcomas and myxoid/round cell liposarcomas were found to have high PD-L1 and PD-1 expression. Future studies are necessary to assess PD-1/PD-L1 as a predictive biomarker in patients with sarcomas.

Among various kinds of immunotherapies, immune checkpoint inhibitors have shown marked anticancer effects on the advanced stages of some cancer types by inhibiting the escape phenomenon of the cancer immune response [58]. Previous studies also suggested that PD-1 and PD-L1 are promising targets for patients with sarcoma. However, there are only a few reports of checkpoint inhibitors for sarcoma (Table 2). Ben-Ami reported that a phase 2 trial of PD-1 inhibition with nivolumab in patients with advanced uterine leiomyosarcoma showed no objective response in 12 patients and grade 3-4 toxicity related to the treatment in 3 patients [59]. On the other hand, Paoluzzi retrospectively investigated the effect of nivolumab in 24 patients with bone and soft tissue sarcoma. The study showed that 3 patients had partial responses, 9 patients had stable disease, and 12 patients had disease progression, and that grade 3-4 toxicity occurred in 5 patients [60].

Ipilimumab, a human monoclonal antibody that inhibits CTLA-4 activity, has been used as an immunotherapy drug and has entered clinical trials for several cancers. In a phase 2 study, 6 patients with synovial sarcoma were treated with ipilimumab every 3 weeks. The endpoint of the study was response rate and secondary endpoints included determination of the clinical benefit rate and evaluation of NY-ESO-1-specific immunity. In the study patients, the progression-free survival period ranged from 0.5 to 2.1 months, and neither clinical response nor evidence of serological or delayed type hypersensitivity to NY-ESO-1 was observed [61].

Immune checkpoints may be a target of immunotherapy in patients with sarcomas. Although immune checkpoint inhibitors have shown marked effects in several cancer types, previous clinical

studies showed no significant clinical benefit in patients with sarcomas. Further studies on the immune response pathway and the indication of checkpoint inhibitors are demanded for improvement of the treatment of bone and soft tissue sarcoma.

Future perspective

Immunotherapy can be considered as the candidate of standard treatment including surgery, chemotherapy, and radiation therapy. Although the effect of cellular immunotherapy is seen in only a part of patients, it is a feasible, well-tolerated, and promising approach in the treatment of children with advanced sarcomas. Recent clinical and basic studies suggest that checkpoint inhibitors are promising treatments for advanced sarcomas, whereas checkpoint inhibitors have shown only a small benefit and severe side effects in some study patients. Most immunotherapies require a large cost and a technically difficult process, and the effect of immunotherapy can be observed in only a limited number of patients. To apply effective immunotherapies in the future, factors associated with immune responses should be investigated to estimate the response to immunotherapy in each patient. Furthermore, further exploration of innovative approach of combination therapy using multimodality immunotherapy is demanded to enhance their antitumor activity.

Executive summary

- Novel therapeutic modalities have been demanded to improve outcome of patients with bone and soft tissue sarcomas. Recent elucidation of the relationships between cancer cells and immune systems has contributed to the development of immunotherapy.
- Immunotherapies, such as vaccinations, cytokine therapies, cellular immunotherapies, and immune checkpoint inhibitors have been investigated for bone and soft tissue sarcomas. However, the effect of immunotherapy can be observed in only a limited number of patients.

- Further studies on the immune response pathway and the indication of immunotherapies are demanded for improvement of the treatment of sarcomas.

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Table 1. Clinical trials using cellular immunotherapy for sarcomas.

Cell therapy	Number of patients	Diagnosis	Treatment	Ancillary treatment	Severe adverse event	Result
TL-pulsed DCs [Geiger, 2001]	15	Pediatric solid tumors	DCs pulsed with TL/KLH (1×10^6–1×10^7 cells)	None	None	PR: 1 SD: 5 PD: 9
TL-pulsed DCs [Suminoe, 2009]	5	EWS (1), SS (1), neuroblastoma (3)	DCs pulsed with tumor antigen and KLH (3×10^6–1.5×10^7 cells)	None	None	CR: 1 SD: 2 PD: 2
TL-pulsed DCs [Himoudi, 2012]	13	OS	DCs pulsed with TL/KLH (1×10^5–1×10^6 cells)	IL-2	None	No tumor regression
TL-pulsed DCs [Miwa, 2017]	37	Sarcoma	DCs pulsed with TL/OK-432/TNF-α (5×10^6 cells)	None	None	PR: 1 SD: 6 PD: 28
Autologous lymphocyte and TL/KLH-pulsed DCs [Merchant, 2016]	Immunotherapy (29), nonimmunotherapy (14)	ES, RMS, DSRCT, SS, and undifferentiated sarcoma	Autologous lymphocyte infusion and DC pulsed with TL/KLH	Standard antineoplastic therapy	Transaminitis (grade 2: 24%; grade 3: 7%) and anaphylaxis (grade 4: 14%) attributed to CYT107	5-year OS: 62% and 29% in patients with or without immunotherapy
HER2-CAR T cells [Ahmed, 2015]	19	OS (16), EWS (1), PNET (1), DSRCT (1)	HER2-CAR T cells (1×10^4–1×10^8/m^2)	None	None	SD: 4 of 17
NY-ESO-1 TCR T cells [Robbins, 2015]	38	Melanoma (20), SS (18)	NY-ESO-1 TCR T cells (0.9×10^{10}–1.3×10^{11} cells)	IL-2	Neutropenia and thrombocytopenia associated	CR: 5/37 PR: 17/37

					with IL2 (100%)	

DC: dendritic cell, TL: tumor lysate, PR: partial response, SD: stable disease, PD: progression of disease

Table 2. Clinical trials using checkpoint inhibitors for sarcoma.

Agent	Number of patients	Diagnosis	Treatment	Severe adverse events	Responses
Anti-PD-1 antibody [Paoluzzi, 2016]	24	Sarcoma	nivolumab (3 mg/kg), every 2 weeks	Elevation of AST/ALT/ALP and bilirubin elevation (1), ALT elevation (1), AST/ALT elevation (1), and pneumonitis, colitis (1)	PR: 3 SD: 9 PD: 12
Anti-PD-1 antibody [Ben-Ami, 2017]	12	Uterine leiomyosarcoma	nivolumab (3 mg/kg), every 2 weeks	Abdominal pain (1), serum amylase and lipase increase (1), and fatigue (1)	CR/PR: 0/12
Anti-CTLA4 antibody [Maki, 2013]	6	Synovial sarcoma	Ipilimumab (3mg/kg), every 3 weeks	Vomiting and diarrhea (1)	PD: 6/6