Adoptive cell therapy for sarcoma

Current therapy for sarcomas, though effective in treating local disease, is often ineffective for patients with recurrent or metastatic disease. To improve outcomes, novel approaches are needed and cell therapy has the potential to meet this need since it does not rely on the cytotoxic mechanisms of conventional therapies. The recent successes of T-cell therapies for hematological malignancies have led to renewed interest in exploring cell therapies for solid tumors such as sarcomas. In this review, we will discuss current cell therapies for sarcoma with special emphasis on genetic approaches to improve the effector function of adoptively transferred cells.

Keywords: cancer immunotherapy • cell therapy • gene transfer • sarcoma

Sarcomas are a diverse group of malignancies that include osteosarcoma (OS), Ewing's sarcoma (EWS), rhabdomyosarcoma (RMS) and nonrhabdomyosarcoma soft-tissue sarcomas, such as synovial sarcoma or desmoplastic small round cell tumors. While patients with local disease have an excellent outcome, the prognosis of patients with advanced stage disease remains poor [1]. Cell therapy in the form of high-dose chemotherapy with autologous stem cell rescue has been extensively explored for sarcomas. However, most studies have not shown a significant survival benefit in comparison to standard chemotherapy, indicating that more specific cell therapies are needed to improve outcomes [2,3].

Recent successes in the field of cellular therapies for hematological malignancies have rekindled efforts to use adoptive cell therapies to treat solid tumors including sarcomas. For example, adoptive transfer of T cells, genetically modified to express NY-ESO-1-specific T-cell receptors (TCRs), resulted in significant antitumor effects in patients with synovial sarcoma [4]. However, cell therapy for sarcoma faces several obstacles, which need to be overcome before these therapies can be introduced into broader clinical practice. What tumor-associated

antigens (TAAs) should we target? What is the best cell product? Do cells have to be genetically modified to enhance their effector function? Do cells have to be combined with other treatment modalities? While there is insufficient information to answer these questions, we will review here the current state of cell therapies for sarcoma with these questions in mind.

TAAs expressed in sarcomas

Although sarcomas are heterogeneous and vary in clinical presentation and biology, various subtypes express common TAAs that should allow the development of cellular therapies that target a broad range of sarcomas. TAAs are commonly divided into antigens that: contain novel peptide sequences created by gene fusions or mutations; are expressed at higher than normal levels in tumor tissues; and/or are normally expressed during fetal development or at immunoprivileged sites, such as the testes. For sarcomas, several TAAs have been described that are summarized in **Table 1**. These include novel fusion proteins such as EWS-FLI-1 expressed in EWS or SYT-SSX2 expressed in synovial sarcoma, which have been targeted using dendritic cells (DC) based vaccines [5,6]. Over-

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expressed antigens such as HER2, IL-11R α , GD2 (a disialoganglioside), CLUAP1, papillomavirus binding factor, FAP, TEM1, B7-H3 and IGF-1R are present in a variety of sarcomas and a few are being targeted by genetically modified cells that avoid the antigen processing/presenting machinery [7–23]. Additionally, cancer testes antigens such as NY-ESO-1, members of the MAGE and GAGE family or developmental antigens such as the fetal acetylcholine receptor are actively being investigated due to the restricted expression [24–30]. Last, sarcomas also express NKG2D ligands as potential targets for cell therapies [31–33]. Since TAA expression in most solid tumors including sarcoma is heterogeneous, TAA expression has to be confirmed for individual patients and effective cell therapy products most likely have to target multiple TAAs to prevent immune escape.

Since results from recent T-cell immunotherapy trials have highlighted potential ‘on target/off cancer’ side effects [34–36], there is a continued need to discover novel TAAs, which are differentially expressed in tumors. It might be impossible to completely prevent

such ‘on target/off cancer’ side effects. While conventional T cells are only able to recognize single antigens, genetic approaches are being developed (see section ‘Preventing toxicities’) that restrict full T-cell activation to the presence of multiple antigens. T cells that recognize ‘antigen addresses’ should not only increase safety, but also reduce the inevitable risk of immune escape when single antigens are targeted.

Cell therapies for sarcomas

DCs, NK cells, tumor infiltrating lymphocytes (TILs), $\gamma\delta$ T cells and genetically modified T cells are actively being explored as therapeutics for sarcoma.

Dendritic cells

The use of DCs is an attractive approach for adoptive cell therapy since they are potent APCs to activate and expand TAA-specific T cells. While this review is focused on ‘none DC-based’ adoptive cell therapies, we will briefly review the clinical results of DC vaccines for sarcoma. Vaccines for sarcoma have been recently reviewed elsewhere [37,38]. Vaccinations with *ex vivo* generated DCs rely on the generation of DCs from patients’ peripheral blood monocytes in the presence of GM-CSF and IL4. DCs are then activated and loaded with TAA-derived peptides, TAAs, tumor lysates or are genetically modified to express TAAs prior to injection [39]. While the US FDA approval for Sipuleucel-T for the treatment of prostate cancer has garnered renewed interest in vaccinations with *ex vivo* matured DCs [40], the clinical antitumor activity of DC-based vaccines in general has been limited. DC-based vaccine studies have been conducted with sarcoma patients and are summarized in Table 2 [41–48]. For example, in one clinical study, 15 pediatric patients with relapsed solid tumors who had failed standard salvage therapies were vaccinated with DCs loaded with autologous tumor lysate and adjuvant (keyhole limpet hemocyanin) [46]. Six out of ten evaluable patients had an increase of their cellular immune response to keyhole limpet hemocyanin and three had a greater than tenfold increase in their cellular immune response to tumor lysate as judged by IFN- γ Elispot assays. Out of these three patients, one had a partial response and the two had stable disease. The overall response rate (stable disease and partial response) was 40%, which is similar to response rates observed on other DC vaccine trials. DC-based vaccines have also been evaluated for sarcoma patients as consolidation or adjuvant therapy [42,48,44]. While these studies demonstrated feasibility and safety, clinical benefit was difficult to ascertain since they were Phase I/II studies and lacked controls that did not receive the vaccine. Clearly, the antitumor activity of DC vaccines needs to be improved. Strategies include enhancing DCs by

Table 1. Tumor-associated antigens expressed in sarcoma.		
Antigen	Sarcoma	Ref.
Unique fusion proteins		
EWS-FLI-1	EWS	[5]
SYT-SSX2	SS	[6]
Overexpressed antigens		
HER2	OS	[7,8]
IL11R α	OS	[9,10]
GD2	EWS, OS	[11,12]
FAP	OS	[13,14]
Papilloma virus binding factor	OS	[15]
IGF-1R	EWS, OS, RMS, SS	[16–19]
B7H3	OS, RMS	[20]
CLUAP1	OS	[21]
STEAP1	EWS	[22]
TEM1	OS, EWS, RMS, SS	[23]
Developmental/cancer testis antigens		
NY-ESO-1	SS	[24,25]
SSX family	OS	[26]
BAGE family	RMS	[27]
GAGE family	RMS	[27]
MAGE family	OS, RMS, SS	[24,27,28]
XAGE family	EWS	[29]
fAChR	RMS	[30]
EWS: Ewing’s sarcoma; OS: Osteosarcoma; RMS: Rhabdomyosarcoma; SS: Synovial sarcoma.		

Table 2. Clinical experience with cell therapy for sarcoma.			
Cell therapy	Disease	Ancillary therapy	Comment
Peptide-loaded DC [41]	EWS (1), SS (1)	None	No toxicities; one CR (EWS; +77 months)
Peptide-loaded DC [42]	EWS (20), RMS (10)	±IL2	As consolidation, 25% of HLA-A2+ patients developed specific immune responses. 43% 5-year overall survival
Peptide-loaded DC [43]	SS (1)		Transient decrease in growth rate of tumor
Peptide-loaded DC/monocytes [44]	EWS (10), PNET (2), RMS (3)	IL2	Eleven had a PR after one cycle; one patient had mixed response after 2nd cycle
Auto tumor lysate-loaded DCs [45]	OS (13)	IL2	No toxicities; no clinical benefit
Auto tumor lysate-loaded DCs [46]	FS (1), PNET (2), OS (1), IMS (1), HS (1), DS (1), EWS (2), CCS (1), other solid tumor (5)	None	No toxicities; nine PD, five SD, one PR
Auto tumor lysate-loaded DCs [47]	DS (1), EWS (5), FS (1), OS (7), other solid tumor (8)	None	No toxicities; patients with active disease (13): one SD, one mixed response; patients treated in CR: 7/9 remained in CR
Auto tumor lysate-loaded DCs	Thirty-two patients with NB, EWS or RMS	Autologous lymphocytes ± IL7	Completed enrollment, results not yet published
DCs directly injected into tumors [48]	Soft tissue sarcoma of the extremities (17)	In combination with local XRT	Preliminary results: nine patients developed specific immune responses; four patients were disease free at 3 years
NY-ESO-1 TCR T cells [4]	SS (6)	IL2	No toxicities; four PR (up to 18 months); two PD
HER2-CAR T cells [112]	OS (16), EWS (2), DSCRT (1)	None	No toxicities, in progress, results not yet published

Auto: Autologous; CAR: Chimeric antigen receptor; CCS: Clear cell sarcoma; CR: Complete response; DC: Dendritic cell; DS: Desmoplastic sarcoma; DSCRT: Diffuse small cell round tumor; EWS: Ewing's sarcoma; FS: Fibrosarcoma; HS: Hepatic sarcoma; IMS: Inflammatory myofibroblastic sarcoma; OS: Osteosarcoma; NB: Neuroblastoma; PD: Progressive disease; PNET: Peripheral neuroectodermal tumor; PR: Partial response; RMS: Rhabdomyosarcoma; SD: Stable disease; SS: Synovial sarcoma; TCR: T-cell receptor; XRT: Radiation therapy.

genetic modification to increase their function [49,50], or combining DC vaccination with the administration of cytokines, chemotherapeutic agents that either deplete inhibitory T cells or upregulate TAA expression in tumor cells, or monoclonal antibodies (mAbs) that deplete inhibitory, regulatory T cells (Tregs) or block immune-cell-intrinsic checkpoints such as CTLA-4 or PD1/PD-L1 (see section 'Combining cell therapies with other therapies').

NK cells

As part of the first line of defense against pathogens, NK cells can recognize nonself cells without prior sensitization or identification of antigens. Instead, NK cells control their cytotoxic function against virally infected or cancerous cells by a balance between inhibitory and activating signals provided through several

surface receptors. Activation of NK cells is initiated when activation receptors, such as NKG2D, DNAM-1 and natural cytotoxicity receptors, such as NKp30, NKp44 and NKp46, recognize their respective ligands on the target cell. In addition, the NK cell must also lack inhibitory signals. These inhibitory signals are typically provided by engagement of killer-cell immunoglobulin-like receptors and MHC class I molecules and serve as a mechanism to distinguish between self and nonself [51,52]. Since NK cells do not require the identification of TAAs to trigger a response, they are a prime candidate for cellular immunotherapy against a variety of cancers including sarcomas.

NK cells have been shown to target EWS, OS and RMS cell lines *in vitro* [31,53,54]. Additionally, a decrease in inhibitory ligands (e.g., MHC class I), and an upregulation of activation receptor ligands

(e.g., MIC and ULBP family members), have been documented in various sarcoma tumor models highlighting the potential therapeutic benefit of NK cells for sarcoma [55,56,33,57]. However, autologous NK cells from cancer patients are often functionally impaired, in addition to being sensitive to inhibitory killer-cell immunoglobulin-like receptors. For example, NK cells of cancer patients often express low levels of activating receptors or intracellular signaling molecules, preventing proper NK-cell function [58]. In an analysis of NK cells from EWS patients, cytolytic function was impaired compared with age-matched controls despite similar expression patterns of the activation receptors NKG2D and DNAM-1 and constant levels of inhibitory ligands on tumor cells [31].

To overcome these road blocks, investigators have expanded NK cells derived from healthy donors *ex vivo* with artificial APCs, K562, which express CD137L and membrane-bound IL15 [59,60] or CD137L and membrane-bound IL21 [61]. Expanded allogeneic NK cells had potent antitumor effects against several malignancies in preclinical studies including EWS and RMS [32]. Currently, two clinical trials are in progress for cancer patients including sarcomas (Table 3). In one trial, patients receive allogeneic NK cells post matched-related or unrelated donor stem cell transplant (NCT01287104), and in the other trial patients receive haplo-identical NK cells derived from a related donor (NCT00640796).

Besides NKG2D ligands, NK cells are also able to recognize other stress signals expressed by tumor cells. For example, tumors aberrantly express heat shock protein (Hsp) 70 on their cell surface, which renders them sensitive to NK cells that recognize an N-terminal HSP peptide [62]. Based on enhanced cytotoxicity and recognition of tumor cells in preclinical testing [63], a Phase I clinical trial was performed with *ex vivo* HSP70-activated NK cells in patients with colorectal and lung carcinoma patients [64,65]. While infusions were well tolerated, limited antitumor effects were observed. Nevertheless, since HSP70 is expressed in OS and chondrosarcoma [66], NK cells recognizing N-terminal HSP70 represent one future cell therapy option for sarcoma.

To enhance the therapeutic potential of NK cells investigators are also pursuing other strategies. These include the genetic modification of NK cells with chimeric antigen receptors (CARs) [67,68], co-infusing NK cells with mAbs [69] or arming NK cells with bispecific NK-cell engagers [70].

Tumor infiltrating lymphocytes

The ability of TILs to home and infiltrate tumors is an attractive feature since other T cells might not express the appropriate chemokine receptors to traffic to tumor sites [71]. The adoptive transfer of *ex vivo* expanded TILs has been predominately explored for patients with melanoma [72], and combining TIL transfer with lymphodepleting chemotherapy and

Table 3. Ongoing cell therapy clinical trials for sarcoma.				
Cell therapy	Sarcoma	Phase	clinicaltrial.gov identifier	Center
Dendritic cells				
DCs pulsed with tumor lysate plus gemcitabine	Sarcoma	I	NCT01803152	University of Miami
DCs pulsed with tumor peptide plus decitabine	EWS, RMS, SS	I	NCT01241162	University of Louisville
NK cells				
Haploidentical donor-derived NK cell infusion	Solid tumors including EWS and RMS	I	NCT00640796	St Jude Children’s Research Hospital
Allogeneic SCT followed by NK cell infusion	Solid tumors including EWS and RMS	I	NCT01287104	NCI
Genetically modified T cells				
HER2-CAR T cells	OS	I	NCT00902044	Baylor College of Medicine
GD2-CAR T cells	Sarcoma	I	NCT02107963	Baylor College of Medicine
GD2-CAR VZV-specific T cells	Sarcoma	I	NCT01953900	NCI
NY-ESO-1 TCR	SS	I	NCT01343043	University of Pennsylvania
CAR: Chimeric antigen receptor; DC: Dendritic cells; EWS: Ewing’s sarcoma; OS: Osteosarcoma; RMS: Rhabdomyosarcoma; SCT: Stem cell transplant; SS: Synovial sarcoma; TCR: T-cell receptor; VZV; Varicella zoster virus.				

radiation has resulted in impressive clinical responses [73,74]. In addition, TIL-derived T-cell clones have been used to clone TAA-specific $\alpha\beta$ TCRs [75]. In contrast to melanoma, isolating and expanding TILs *ex vivo* from other tumor types including sarcomas is not very reliable. Nevertheless, TILs have been successfully extracted from small tumor fragments and cultured *ex vivo* in the presence of IL2 from EWS, OS, RMS and giant cell tumors [76–78]. TIL cultures contained a variable ratio of CD4- and CD8-positive T cells [76], and these TILs had higher lytic activity against tumor cells in comparison to peripheral blood lymphocytes. Due to the unreliable isolation and expansion of TILs from sarcoma samples, and the recent advances of genetic modification strategies to rapidly generate TAA-specific T cells, it is unlikely that TILs will be evaluated in clinical trials for sarcoma patients. However, several studies suggest that the presence of TILs in sarcomas correlate with outcome [79]. Thus, detailed analysis of TILs in sarcoma has the potential to reveal important insights into immune evasion mechanisms of sarcoma. In addition, determining the antigen specificity of the TILs might identify TAAs that could be targeted with other T-cell therapy approaches.

$\gamma\delta$ T cells

The use of $\gamma\delta$ T cells in cancer immunotherapy is an emerging field, especially in the context of sarcomas. $\gamma\delta$ T cells, specifically V γ 9V δ 2, account for a small percentage of the T-cell population in the peripheral blood and possess a unique TCR containing both the γ and δ glycoprotein chains instead of the typical α and β chains. Unlike $\alpha\beta$ T cells, $\gamma\delta$ T cells can recognize unprocessed antigens in an MHC-independent manner [80]. This is advantageous since tumors are often resistant to $\alpha\beta$ T-cell-mediated cytotoxicity due to downregulation of MHC molecules on the tumor surface. Instead, $\gamma\delta$ T cells recognize nonpeptide, phosphoantigens by germline-encoded regions of the TCR [81]. Additionally, these cells express NKG2D and can therefore be activated via engagement of NKG2D ligands, which would also be advantageous in the context of sarcomas because of their NKG2D ligand expression [82].

$\gamma\delta$ T cells can be harnessed for cancer immunotherapy by two different immunotherapeutic strategies: adoptive transfer of *ex vivo* expanded $\gamma\delta$ T cells, or administration of $\gamma\delta$ TCR agonists, such as aminobisphosphonates, which not only activate $\gamma\delta$ T cells but also have direct antitumor effects by preventing bone metastasis and inhibiting tumor cell proliferation [83,84]. Adoptive transfer of $\gamma\delta$ T cells for patients with metastatic renal cell carcinoma has shown promising clinical benefits [85,86]. In one patient, complete

remission was achieved after several rounds of $\gamma\delta$ T-cell infusions and maintained over 3 years at the time of publication [87]. Activation of $\gamma\delta$ T cells *in vivo* by the administration of aminobisphosphonates has also shown clinical responses in breast cancer [88], prostate cancer [89] and renal cell carcinoma [90].

While these studies show the potential use of $\gamma\delta$ T cells to treat sarcoma, only preclinical studies using OS cell lines have been done so far [91,92]. *Ex vivo* activated $\gamma\delta$ T cells kill OS cell lines *in vitro* through several mechanisms including granzyme/perforin and Fas/Fas-ligand interaction [93]. These studies highlight the potential antisarcoma activity of $\gamma\delta$ T cells and provide rationale to evaluate $\gamma\delta$ T cells in clinical studies in the future, which should be feasible due to the recent advances in ‘clinical grade’ *ex vivo* expansion of $\gamma\delta$ T cells [94].

Genetically modified T cells

While virus-specific T cells can be readily activated and expanded *ex vivo*, the activation and expansion of clinical grade TAA-specific T cells is cumbersome. To overcome this limitation, T cells have been genetically modified to express one of two receptors that redirect their specificity toward tumor cells. First, cells can be modified to express transgenic TCRs that recognize peptides derived from TAAs presented by MHC molecules, thereby taking advantage of the T cells’ natural ability to recognize antigens. Second, T cells can also be modified to express artificial receptors, called CARs, which recognize TAAs in their unprocessed form on the cell surface of tumor cells. This approach now enables the T cell to react to antigens that would normally not have been seen by the cells’ conventional antigen recognition mechanism. In addition, gene transfer is a promising approach to improve the effector function of adoptively transferred cells (Figure 1).

Transgenic $\alpha\beta$ TCR

To generate TAA-specific TCR T cells, genes encoding α and β chains of the TCR complex are genetically introduced into T cells [95]. Several HLA-A2-restricted TCRs have been cloned that recognize TAAs, including MART-1 [96], gp100 [97], MAGE-A3 [98] and NY-ESO-1 [99]. The majority of isolated TAA-specific TCRs are of low affinity and require additional affinity maturation to increase their ability to recognize TAA-expressing tumor cells [100]. While effective, this can result in the recognition of other antigens with fatal consequences as recently documented by MAGE-A3 TCR T cells that also recognized titin expressed in cardiac muscle [101]. Besides ‘low affinity,’ misspairing of the introduced α and β chains with endogenous α and β TCR chains is another obstacle, which not only

Figure 1. Genetic modifications to improve T-cell therapies. T cells can be genetically modified to (A) render T cells specific, (B) enhance their expansion, (C) render them resistant to the immunosuppressive tumor microenvironment and (D) enhance their safety. Brown: T cell; Blue: Tumor cell. For details see text. TCR: T-cell receptor.

renders the introduced TCR ineffective, but could also result in 'novel specificities' thereby increasing the risk of off-target toxicities. Investigators have developed several approaches to favor pairing of the introduced α and β chains, including the introduction of disulfide bonds or the use of murine TCR transmembrane

domains [102,103]. Other strategies include the silencing of endogenous TCR expression or simply expressing the TCRs in $\gamma\delta$ T cells [104,105].

Despite these obstacles, several clinical studies have been conducted with TCR T cells. MART-1-, gp100- or NY-ESO-1-specific TCR T cells have been evalu-

ated in melanoma patients with encouraging antitumor responses in small number of patients [4,106,107]. In addition, synovial sarcoma patients have received NY-ESO-1-specific TCR T cells [4]. Four out of six patients had objective clinical responses with no adverse toxicities, and in one patient, the antitumor response lasted for 18 months. Although the use of transgenic TCR T cells in treating sarcoma patients is limited, based on the encouraging results with NY-ESO-1-specific TCR T cells, a followup study for synovial sarcoma patients is in progress (NCT01343043).

Chimeric antigen receptors

CARs engrafted onto T cells are emerging as a promising therapeutic strategy in a variety of malignancies, and currently a large number of clinical trials are actively investigating the safety and efficacy of CAR T-cell therapy in humans with special focus on CD19-positive hematological malignancies [108,109]. CARs consist of an extracellular antigen recognition domain, a hinge, a transmembrane domain and an intracellular signaling domain. The extracellular antigen recognition domain most commonly consists of a single chain variable fragment, less frequently peptides or ligands have been used. Different hinges, long or small, have been evaluated, and recent studies indicate that the hinge is not only a structural component of CAR, but greatly influences its function. Commonly used transmembrane domains include the transmembrane domain of CD28 or CD8 α . While 'original CARs' only contained the CD3 ζ chain as an endodomain (first-generation CARs), signaling domains of co-stimulatory molecules such as CD28, 41BB, OX40 or ICOS have been added to enhance CAR function. Depending on the number of added co-stimulatory domains, these CARs are referred to as second generation (one co-stimulatory endodomain) or third generation (two co-stimulatory endodomains).

Several groups have explored the use of second- and third-generation CAR T cells to treat sarcomas in preclinical models targeting HER2, IL-11R α , NKG2D and fetal acetylcholine receptor [8,10,110,111]. However, limited clinical experience is available (Tables 2 & 3). Safety concerns have been raised in regards to targeting HER2 with CAR T cells in humans due to one patient who died of respiratory failure after receiving 1×10^{10} T cells expressing a third-generation HER2-specific CAR with IL2 after high-dose chemotherapy [34]. Subsequently, in a separate clinical study, up to $1 \times 10^8/\text{m}^2$ T cells expressing a second-generation CAR were given to pediatric and adolescent sarcoma patients. While the infusions were safe, infused T cells did not expand significantly postinfusion, and antitumor activity of the infused T cells was limited [112].

These results highlight the need for a balance between optimal CAR T-cell expansion, persistence and safety in future clinical studies (see section 'Improving cell therapies for sarcomas'). CARs targeting other TAAs in sarcoma are also actively being evaluated in the clinical setting. Based on encouraging clinical data using T cells expressing a first-generation GD2-specific CAR in neuroblastoma patients [113,114], two clinical studies are in progress that evaluate the safety and efficacy of third-generation GD2-specific CAR T cells in patients with sarcoma (NCT01953900, NCT02107963).

Improving cell therapies for sarcomas

Sarcomas share several obstacles of cell therapies with other solid tumors. These include: potential on target/off cancer toxicities; limited T-cell expansion; and the immunosuppressive tumor microenvironment.

Preventing toxicities

Although sarcomas express unique fusion genes or developmental restricted antigens, the majority of cell surface TAAs are also expressed at low levels in normal tissue. To prevent toxicity of infused cell products, two approaches are actively being pursued. One is the introduction of suicide genes, which can be used to selectively kill genetically modified cells in the event of side effects. The other relies on genetically engineering T cells to limit their activation to tumor sites.

Suicide genes

Three classes of suicide genes are at various stages of clinical development [115]. The first class relies on the expression of an enzyme that activates a prodrug into a toxic compound. The most widely used gene is the herpes simplex virus thymidine kinase (HSV-*tk*) that converts acyclovir or ganciclovir into toxic compounds. For example, clinical studies with HSV-*tk*-transduced T cells post stem cell transplantation have shown that administration of ganciclovir (GCV) efficiently ablates HSV-*tk*-transduced T cells *in vivo* [116]. The second class consists of genes that can be specifically activated to induce T-cell apoptosis. For example, T cells that express an inducible caspase 9 gene can be effectively ablated in preclinical models and in patients by administration of a 'dimerizer' [117,118]. Last, expression of a cell surface antigen on T cells such as truncated CD20 or EGFR allows the elimination of T cells with FDA-approved mAbs [119,120].

Engineering of T cells to limit their activation to tumor sites

While TAA discovery is actively being pursued, it might be impossible to discover single antigens that are uniquely expressed in sarcomas and not expressed at low levels in

normal tissues. However, tumors most likely express a unique pattern of antigens, which can be exploited using genetically modified T cells. For example, T cells have been engineered to express two CARs with different antigen specificity. One CAR provides antigen-specific ζ -activation, while the second CAR antigen-specific co-stimulation, restricting full T-cell activation to tumors, which expresses a 'unique TAA address' [121–123]. In addition to targeting multiple TAAs on tumor cells, expressing inhibitory CARs in T cells that inhibit T-cell function upon antigen recognition on normal cells is another strategy to prevent 'on target/off cancer' toxicities [124]. Last, T cells can be modified with a first-generation CAR and chimeric cytokine receptors to restrict full T-cell activation to tumor sites (see section 'Counteracting the immunosuppressive tumor environment').

Enhancing T-cell expansion

Significant expansion of adoptively transferred T cells in the peripheral blood has only been observed in patients post stem cell transplantation or in patients who received lymphodepleting chemotherapy and/or radiation prior to T-cell transfer [125,73]. While effective, lymphodepleting chemotherapy and/or radiation can be associated with unwanted toxicities. Lymphodepleting mAbs are one attractive strategy to replace chemotherapy. For example infusion of a pair of rat CD45 mAbs, which have a short half-life in humans, prior to T-cell transfer, induced transient lymphodepletion resulting in enhanced, albeit limited expansion of adoptively transferred T cells [126]. While the infusion of the humanized CD52 mAb alemtuzumab induces profound lymphodepletion [127], it was deemed unsuitable to aid T-cell expansion, since the infused T cells express CD52 and alemtuzumab has a half-life of approximately 21 days. However, preclinical studies indicate that T cells in which CD52 expression is silenced, readily expand post CD52 mAb infusion, opening the opportunity to combine alemtuzumab with T-cell transfer in clinical trials in the future [128].

Genetic strategies to enhance T-cell expansion without lymphodepletion are also actively being explored. For example, the transgenic expression of IL15 has been shown in preclinical models to promote survival and expansion of gene-modified cells [129,130]. In addition, the expression of the IL7R α in combination with IL7 administration, has been shown to enhance T-cell expansion [131,132]. In such an approach, T cells that were once unresponsive to IL7 due to the lack of the cytokine receptor are now able to receive survival and proliferation signals induced by IL7. Last, recent studies have highlighted that certain T-cell subsets might have enhanced survival *in vivo*. T cells derived from the central memory pool have shown superior expansion and persistence *in vivo* compared with bulk T cells [133,134].

Counteracting the immunosuppressive tumor environment

Malignant cells of solid tumors including sarcomas and their supporting stroma have developed an intricate system to suppress the immune system [135–138]. This includes the expression of cell surface molecules such as PD-L1 and FAS ligand on tumor cells and the secretion of cytokines such as TGF- β or IL10. In addition, malignant and stromal cells attract immunosuppressive cells such as myeloid derived suppressor cells and Tregs to further dampen the function of tumor-reactive T cells [139]. Several genetic modification strategies have been developed to render T cells resistant to this hostile environment, including silencing of inhibitory genes expressed by tumor cells, transgenic expression of dominant negative receptors, 'signal converters' and transgenic expression of secretable cytokines. For example, silencing Fas in T cells by using siRNA prevented FAS-induced apoptosis in the presence of FAS-ligand expressing tumors [140]. Such an approach has the potential to improve T-cell function and could be applied to immunotherapeutic approaches against sarcomas.

In addition to several other cytokines secreted by tumors, TGF- β is a widely used tumor immune evasion strategy since it can promote tumor growth while drastically decreasing tumor-specific cellular immunity [141,142]. These unfavorable effects of TGF- β can be counteracted by modifying T cells to express a dominant-negative TGF- β type II receptor (DNRII) [143,144]. Results of an ongoing clinical study suggest that DNRII-modified tumor-specific CTLs might benefit patients who failed therapy with tumor-specific T cells [145]. In addition, while dominant negative receptors only protect T cells from the immunosuppressive environment, 'signal converters' convert inhibitory signals into stimulatory signals. For example, linking the extracellular domain of the TGF- β RII to the endodomain of toll-like receptor (TLR) 4 results in a chimeric receptor that not only renders T cells resistant to TGF- β , but also induces T-cell activation and expansion [146]. Similar approaches have also been used to convert inhibitory functions of IL4 into T-cell stimulatory signals [147,148].

As an alternative method to provide stimulatory signals by cytokines, T cells can be genetically modified to secrete cytokines that aid in immune cell function and survival. Transgenic expression of IL15 has been shown to not only enhance CAR T-cell expansion and persistence *in vivo*, but also renders CAR T cells resistant to the inhibitory effects of Tregs [149]. In addition, transgenic expression of IL12 in CAR T cells reverses the immunosuppressive tumor environment and results in enhanced antitumor activity of adoptively transferred T cells in several preclinical animal mod-

els [150,151]. While there are safety concerns in regards to constitutive IL12 expression, IL12 production can be restricted to activated T cells at tumor site by using inducible expression systems [152].

Last, most solid tumors have a stromal compartment that supports tumor growth directly through paracrine secretion of cytokines, growth factors and nutrients, thereby influencing tumor-induced immunosuppression [153]. Therefore, targeting components of the tumor stroma, such as the tumor vasculature or cancer-associated fibroblasts, might be an additional method to counteract the immunosuppressive tumor environment. CARs specific for VEGFR-2 or FAP, in combination with T cells that target tumor cells, has shown to improve antitumor effects, indicating that targeting nonmalignant cells within solid tumors has the potential to improve CAR T-cell therapy approaches for cancer [154,155].

Combining cell therapies with other therapies

As with other areas of cancer therapy, combinatorial therapies will most likely be the key to improving the efficacy of cell therapies for sarcoma. For example, epigenetic modifiers such as HDAC inhibitors can upregulate expression of TAAs resulting in enhanced antitumor effects of T-cell therapies in preclinical models [156,157]. Additionally, small molecule inhibitors that block pathways that are critical for the growth of malignant cells, such as BRAF inhibitors, also enhance the efficacy of adoptively transferred T cells in preclinical models [158]. Last, a promising approach is the use of mAbs that block immune-cell-intrinsic checkpoints and therefore have the potential to enhance adoptive cell therapies given their antitumor activity as single agents. Blocking CTLA-4 on T cells or blocking the interaction between the inhibitory receptor PD-1 and its ligand (PD-L1) on tumor cells can avert inhibitory signals and therefore enhance the function of tumor-specific T cells within the tumor microenvironment. CTLA-4, PD-1 and PD-L1 blocking mAbs have shown promising clinical responses in solid tumors, particularly metastatic melanoma [159–161]. Limited data are available for the treatment of sarcomas using these mAbs. So far, the CTLA-4 mAb, ipilimumab, has only been evaluated in one Phase II clinical study with synovial sarcoma patients. None of the six treated patients developed TAA-specific immune responses or had clinical benefit [162]. Other studies with ipilimumab on which sarcoma patients are included are in progress (e.g., NCT01445379). Since several sarcomas express PD-L1 on their cell surface and sarcoma-infiltrating T cells express PD1 [163,136], it seems advisable to evaluate mAbs that block PD1/PD-L1 in future clinical stud-

ies for sarcoma patients as single agents. In addition, combining these mAbs with adoptive cell therapy holds the promise of enhancing antitumor effects giving encouraging results in preclinical studies [164].

Conclusion

The past 30 years has seen a steady increase in cure rates for patients with localized sarcoma. However, metastatic and/or refractory disease remains largely incurable. Immune-based adoptive cell therapies hold the promise to improve outcomes since they do not rely on the cytotoxic mechanisms of conventional therapies. DC-based vaccines have been explored in several clinical studies for sarcoma. However, while safe, their efficacy has been disappointing. Recent advances in our ability to counteract the immunosuppressive sarcoma environment and to enhance the effector function of immune cells has led to a renewed interest in exploring cell therapies for sarcoma. While early-phase clinical studies with genetically-engineered T cells are in progress, there is a need to further decipher immune evasion strategies of sarcomas and to discover new TAAs as targets for immunotherapy. Giving the ‘increased pace’ of advances in the field of cancer immunotherapy in the last 5 years, we are hopeful that immune-based cell therapies will become an integral part of our therapeutic armamentarium against sarcoma in the future.

Future perspective

Cell therapy for sarcoma has been explored for the last two decades mainly in preclinical models. Recent advances in genetic engineering to render cells not only specific for sarcoma but also resistant to their immune evasion mechanisms holds the promise to improve outcomes for patients with advanced stage disease that fail current therapies. Last, combining cell therapy with other treatment modalities will most likely be critical for their success.

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Executive summary

- Sarcomas differentially express tumor-associated antigens that can be targeted with antigen-specific T cells.
- Since the majority of tumor-associated antigens discovered to date are expressed at low levels in normal tissues, there is the need to discover new antigens and/or engineer T cells to recognize a sarcoma-specific 'antigen address.'
- Dendritic cells, NK cells, tumor infiltrating lymphocytes, $\gamma\delta$ T cells and T cells are actively being explored as therapeutics for sarcoma, however, clinical experience is limited.
- Genetically modifying immune cells has the potential to prevent on- or off-target toxicities; enhance their effector function *in vivo*; and overcome the immunosuppressive tumor microenvironment.
- Combining cell therapies with other targeted therapies holds the promise to enhance their antitumor activity.

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