REVIEW

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Immunotherapy of sarcomas with modified T cells

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Purpose of review
To summarize the development of modified T-cell therapies in sarcomas and discuss relevant published and ongoing clinical trials to date.

Recent findings
Numerous clinical trials are underway evaluating tumor-specific chimeric antigen receptor T cells and high affinity T-cell receptor (TCR)-transduced T cells in sarcomas. Notably, translocation-dependent synovial sarcoma and myxoid/round cell liposarcoma are the subject of several phase II trials evaluating TCRs targeting cancer testis antigens New York esophageal squamous cell carcinoma-1 (NY-ESO-1) and melanoma antigen-A4 (MAGE A4), and response rates of up to 60% have been observed for NY-ESO-1 directed, modified T cells in synovial sarcoma. Challenges posed by modified T-cell therapy include limitations conferred by HLA-restriction, non-immunogenic tumor microenvironments (TME), aggressive lymphodepletion and immune-mediated toxicities restricting coinfusion of cytokines.

Summary
Cellular therapy to augment the adaptive immune response through delivery of modified T cells is an area of novel therapeutic development in sarcomas where a reliably expressed, ubiquitous target antigen can be identified. Therapeutic tools to improve the specificity, signaling, proliferation and persistence of modified TCRs and augment clinical responses through safe manipulation of the sarcoma TME will be necessary to harness the full potential of this approach.

Keywords
chimeric antigen receptor T, melanoma antigen, myxoid, New York esophageal squamous cell carcinoma-1, sarcomas, synovial, T-cell, T-cell receptor

INTRODUCTION

Soft tissue (STS) and bone sarcomas are a heterogenous group of rare mesenchymal malignancies, with over 100 recognized subtypes, accounting for 1% of adult cancers [1]. Approximately 50% of STS patients with intermediate-grade or high-grade disease develop metastasis, and standard first-line treatment remains doxorubicin-based chemotherapy [2]. Median overall survival (OS) for advanced STS is 14–19 months, with overall response rates to doxorubicin approximately 20% [3–5]. Low disease control rates and limited durability of responses has motivated the exploration of a variety of novel immunotherapeutic approaches. These strategies have evolved as cytotoxic properties of T cells become the focus of efforts to harness the immune system against cancer [6]. Limited benefit from immune checkpoint blockade has engendered the development of novel approaches including cellular therapies with modified T cells, modulation of tumor-associated macrophages, cancer vaccines and oncolytic virotherapy [7,8]. Antigen-directed, engineered T-cell receptors (TCRs) offer a therapeutic avenue in sarcomas where a reliable target-antigen can be identified. The greatest potential for success has been observed in synovial sarcoma and myxoid/round cell liposarcoma (MRCL), and the success of New York esophageal squamous cell carcinoma-1 (NY-ESO-1) and melanoma antigen (MAGE) targeted TCRs in phase II trials (Table 1).

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KEY POINTS

- Numerous clinical trials are underway evaluating tumor-specific chimeric antigen receptor T cells and high affinity TCR-transduced T cells in sarcomas.
- Translocation-dependent synovial sarcoma and myxoid/round cell liposarcoma are the subject of several phase II trials evaluating TCRs targeting cancer testis antigens NY-ESO-1 and melanoma antigen-A4, and response rates of up to 60% have been observed for NY-ESO-1 directed, modified T cells in synovial sarcoma.
- Challenges posed by modified T-cell therapy include limitations conferred by HLA-restriction, non-immunogenic TMEs, aggressive lymphodepletion and immune-mediated toxicities restricting coinfusion of cytokines.
- Therapeutic tools to improve the specificity, signaling, proliferation and persistence of modified TCRs and augment clinical responses through safe manipulation of the sarcoma TME will be necessary to harness the full potential of this approach.

IMMUNE CHECKPOINT BLOCKADE IN SARCOMAS

Checkpoint inhibitors (CPIs) have transformed the treatment of several solid malignancies [9–12]. Response to CPI in advanced STS has been varied, with promising activity and durable responses observed in undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, myxofibrosarcoma and angiosarcoma [13–17]. These are more commonly observed alongside a high tumor mutational burden, with exceptions including alveolar soft part sarcoma, where immune cell infiltration is likely associated with the oncogenic *ASPSCR1-TFE3* fusion [15,18–22]. Combination CPI is under evaluation for these subtypes alongside leiomyosarcoma (e.g. phase II Alliance A09140 and NCT04480502), where response to single agent has been poor despite demonstrably high PD-L1 expression [23–25]. CPI in combination with chemotherapy [26] and T-VEC [27] has demonstrated efficacy in advanced sarcomas, and is of particular interest in angiosarcoma (NCT04339738, NCT03921073, NCT03512834) [14,16,21]. The less mutated, translocation-dependent subtypes synovial sarcoma and MRCL are immunologically complex, demonstrating alongside a 'cold' inflammatory phenotype, with low T-cell infiltration and PD-L1/PD-1 expression (predicting poor response to CPIs [23,28]), characteristic cell surface expression of immunogenic cancer testis antigens (CTAs), motivating early phase trials of CTA-specific, engineered TCRs [14,28–30]. Low major histocompatibility complex (MHC) expression may be a mechanism by which synovial sarcoma/MRCL evade the immune system [8], and manipulation of the tumor microenvironment (TME), for example with concurrent vaccine, oncolytic viruses or cytokine infusion, may improve responses to immunotherapies [28]. Systemic interferon gamma (IFNγ) has been demonstrated to drive inflammation and induce MHC and PD-L1 expression, and associated T-cell infiltration in synovial sarcoma/MRCL [31].

ADOPTIVE CELL THERAPY

In solid tumors, adoptive therapies utilizing transfer of *ex vivo* expanded, polyclonal tumor-infiltrating lymphocytes (TILs) have demonstrated efficacy in a number of malignancies including metastatic melanoma and lung cancer [6,32–36]. Generation of TILs for clinical use evolved from the use of lymphokine-activated killer cells, where incubation of lymphocytes with IL-2, generated cells capable of mediating tumor regression [37]. In STS, sufficient expansion of CD3+ TIL cultures with tumor-specific reactivity has led to an active clinical trial [38].

Modified T-cells: Chimeric antigen receptor T cells vs engineered, high affinity T cell receptors

The success of CD19-targeted chimeric antigen receptor (CAR) T-cell therapy exemplifies the ability of adoptive cell therapy (ACT) to induce durable remissions in nonimmunogenic cancers through antigen-directed T-cell cytotoxicity [39–45]. CARs comprise an antigen-binding, extracellular domain coupled to the intracellular signaling CD3ζ domain of a TCR, in addition to costimulatory receptors, and are generated using retroviral transduction or CRISPR–Cas9 technology [6,46]. Generated against extracellular targets, they circumvent MHC restriction and thus can be utilized independent of HLA-haplotype and/or expression. However, a paucity of identifiable targets in solid tumors has resulted in few late-stage trials, although this remains an area of high scientific priority. A phase I/II trial of human epidermal growth factor receptor 2 (HER-2)-directed CAR-T cells in Ewing and osteosarcoma demonstrated persistence of CAR-Ts for several weeks, and some evidence of tumor necrosis [47]. CAR-T trials in sarcomas are summarized in Table 2, and include HER-2-directed and GD2-directed CARs.

High-affinity TCRs are advantageously directed against intracellular, antigen-derived peptides expressed on cancer cell surfaces by MHC molecules,

Table 1. Current clinical trials evaluating high affinity modified T-cell receptors in sarcoma

Trial number	Phase	Condition	Target	Drugs
NCT03462316	I	Bone and STS	NY-ESO-1	Autologous, NY-ESO-1 TCR transduced T cells, lymphodepletion (fludarabine + cyclophosphamide)
NCT02319824	I	Metastatic NY-ESO-1-expressing sarcomas	NY-ESO-1	Autologous NY-ESO-1 TCR-transduced CD8-positive T cells, palliative radiation
NCT03250325	I/II	Unresectable and anthracycline refractory synovial sarcoma	NY-ESO-1	TBI-1301 (autologous NY-ESO-1 TCR-transduced T cells), lymphodepletion (cyclophosphamide)
NCT01343043 completed	I	Metastatic or recurrent synovial sarcoma	NY-ESO-1	Autologous NY-ESO-1c259 TCR-transduced T cells, lymphodepletion (fludarabine, cyclophosphamide)
NCT04318964	I	STS	NY-ESO-1	TAEST16001 cells (autologous, TCR-transduced NY-ESO-1 specific T cells)
NCT03450122	I	Recurrent myxoid/round cell liposarcoma or synovial sarcoma	NY-ESO-1	Autologous NY-ESO-1 TCR-transduced CD8+ T cells, lymphodepletion (cyclophosphamide), aldesleukin ± dendritic cell-targeting lentiviral vector ID-LV305, CMB305
NCT01477021	I	Metastatic or unresectable, anthracycline refractory synovial sarcoma or myxoid/round cell liposarcoma	NY-ESO-1	Autologous NY-ESO-1 specific CD8+ T cells, lymphodepletion (cyclophosphamide)
NCT02869217	I	NY-ESO-1 expressing solid tumors, including synovial sarcoma	NY-ESO-1	TBI-1301 (NY-ESO-1-specific TCR-transduced autologous T cells), lymphodepletion (cyclophosphamide and fludarabine)
NCT03240861	I	Stage IV or locally advanced, unresectable NY-ESO-1-positive cancers	NY-ESO-1	TCR-transduced NY-ESO-1-specific PBMC and CD34+ PBSC, myeloablation (busulfan and fludarabine), aldesleukin
NCT02650986	I/IIa	Advanced sarcoma, melanoma, ovarian cancer	NY-ESO-1	TGFbDNRII-transduced autologous TILs, lymphodepletion (cyclophosphamide)
NCT02992743	II	Advanced myxoid/round cell liposarcoma	NY-ESO-1	Letetresgene autoleucel (GSK3377794, NY-ESO-1 c259 T cells), lymphodepletion (cyclophosphamide and fludarabine)
NCT03967223	II	Advanced synovial or myxoid/ round cell liposarcoma	NY-ESO-1/ LAGE1a	Letetresgene autoleucel (GSK3377794, NY-ESO-1 c259 T cells), lymphodepletion (cyclophosphamide and fludarabine)
NCT04526509	I	Advanced synovial sarcoma and nonsmall cell lung cancer	NY-ESO-1/ LAGE1a	GSK3901961, GSK3845097 (TCR-transduced NY-ESO-1-specific T cells), lymphodepletion (cyclophosphamide and fludarabine)
NCT04044768	II	Advanced synovial and myxoid/ round cell liposarcoma	MAGE-A4	Afamitresgene autoleucel (ADP-A2M4 SPEAR T cells)
NCT03132922	I	Advanced synovial and myxoid/ round cell liposarcoma and other MAGE-A4+ cancers	MAGE-A4	Autologous genetically modified MAGE-A4c1032 T cells combined with low dose radiation

MAGE, melanoma antigen; NY-ESO-1, New York esophageal squamous cell carcinoma-1; PBMC, peripheral blood mononuclear cells; PBSC, peripheral blood stem cells; STS, soft tissue sarcoma; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

increasing potential applicability. Autologous TCR-transduced T cells are generated by viral transduction and administered following fludarabine and cyclophosphamide lymphodepletion (Fig. 1) [48]. The first such cell products evaluated in clinical trials were MART-1 and gp-100 reactive, developed for use in melanoma following observed success in trials of autologous TILs [32–34,49,50].

CANCER TESTIS ANTIGENS

Comprising more than 70 gene families, CTAs are immunogenic cell surface peptides absent in healthy adult tissue apart from the immunoprotected testes [51–53]. Their elicitation of a T-cell mediated immune response was first noted in murine tumor models and later following clinical trials of vaccination with NY-ESO-1 peptide [54,55]. In view of abundant expression in synovial sarcoma/MRCL, CTAs NY-ESO-1, MAGE and Preferentially Expressed Antigen in Melanoma (PRAME) are the subject of developmental T-cell therapies targeting these antigens (Table 1). NY-ESO-1 is a CTA homogenously expressed in synovial sarcoma (70–80% of cases) and MRCL alongside heterogeneous expression in several other cancers [56–61] NY-ESO-1 interacts with MAGE proteins and may be important in cancer cell proliferation and survival through inhibition of p53

Table 2. Current clinical trials evaluating chimeric antigen receptor T cell therapy in sarcoma

Trial number	Phase	Condition	Target	Treatment
NCT04995003	I	Advanced sarcoma	HER2	Lymphodepletion (fludarabine + cyclophosphamide), pembrolizumab or nivolumab, autologous HER2-CAR-T cells
NCT03356782	I/II	Stage III, IV or relapsed sarcoma	Sarcoma-specific	Sarcoma-specific CAR-T cells
NCT00902044	I	Advanced sarcoma	HER2	Autologous HER2-CAR-T cells with or without lymphodepletion (fludarabine or fludarabine + cyclophosphamide)
NCT04433221	I/II	Advanced sarcoma	Sarcoma-specific targets	Sarcoma-specific CAR-T cells
NCT02107963 (complete)	I	GD2+ solid tumors in children and young adults	GD2	Lymphodepletion (cyclophosphamide) + GD2-CAR-T cells
NCT03960060	I	ROR2 positive Stage IV metastatic solid tumors, to include STS	ROR2	Lymphodepletion (fludarabine + cyclophosphamide), CAR-T cells (target not identified, presumably ROR2)
NCT04556669	I	Advanced solid tumors	PDL1, CD22	Autologous aPD-L1 armoured, anti-CD22 CAR-T/CAR-TILs
NCT04897321	I	Pediatric recurrent/refractory B7H3+ solid tumors	B7H3	Lymphodepletion (fludarabine + cyclophosphamide) autologous, B7-H3-CAR T cells
NCT03618381	I	Refractory or recurrent, non-CNS, EGFR + solid tumors	EGFR CD19	Autologous EGFR ± CD19-CAR-T cells
NCT04483778	I	Recurrent/refractor B7H3 solid tumors in young adults	B7H3, CD19	Autologous B7G3 ± CD19-CAR-T cells
NCT04107142	I	Relapsed or refractory solid tumors	NKG2DL	Allogeneic NKG2DL-specific CAR-T cells
NCT04511871	I	Relapsed or refractory stage IV metastatic HER2-positive solid tumors	HER2	Autologous HER2-CAR-T cells
NCT03635632	I	Relapsed or refractory GD2+ solid tumors	GD2	Autologous GD2-CAR-T cells ± lymphodepletion (fludarabine + cyclophosphamide)
NCT04377932	I	Pediatric GPC3-positive solid tumors	GPC3	Lymphodepletion (fludarabine + cyclophosphamide), autologous GPC3-CAR-T cells (IL15 expressing)
NCT04715191	I	Pediatric GPC3-positive solid tumors	GPC3	Lymphodepletion (fludarabine + cyclophosphamide), autologous GPC3-IL15-IL21 CARE-T cells
NCT03721068	I	Relapsed/refractory neuroblastoma or relapsed/refractory osteosarcoma	GD2	Lymphodepletion (fludarabine + cyclophosphamide), autologous GD2-CAR-T cells (IL15, iCas9 expressing)
NCT04539366	I	Recurrent/refractory neuroblastoma and osteosarcoma	GD2	Lymphodepletion (fludarabine + cyclophosphamide), autologous GD2-CAR-T cells

CAR, chimeric antigen receptor; CNS, central nervous system; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; STS, soft tissue sarcoma; TIL, tumor-infiltrating lymphocyte.

[62–65]. The SS18–SSX pathognomonic fusion protein that characterizes synovial sarcoma may mediate epigenetic changes leading to high CTA expression [66,67].

In 2008, the CDR3a and CDR2B amino acid substitutions on the IG4 TCR were identified and noted to enhance the reactivity of TCR-transduced T cells to NY-ESO-1 [68]. These recognized peptide SLLMWITQC, corresponding to amino acid residues 157–165 of NY-ESO-1, and were shown to augment the specificity of recognition of NY-ESO-1∗/HLA-A∗02+ tumor cell lines by TCR gene-modified CD4+ T cells. The anti-NY-ESO-1, SLLMWITQC-specific TCR, named *1G4-α95:LY*, was translated to clinical evaluation in a pilot study in which autologous T cells (CD4+ and CD8+) were retrovirally transduced to encode the TCR, and expanded *in vitro* before adoptive transfer [69]. Overall, 11 of 18 synovial sarcoma patients (61%) with NY-ESO-1 (+) disease (>50% expression) demonstrated objective clinical responses, with 3 and 5 years survival rates of 38 and 14% [70]. Two patients exhibited durable complete responses, the longest nearly 4 years, and partial responses lasted from 3 to 18 months. The

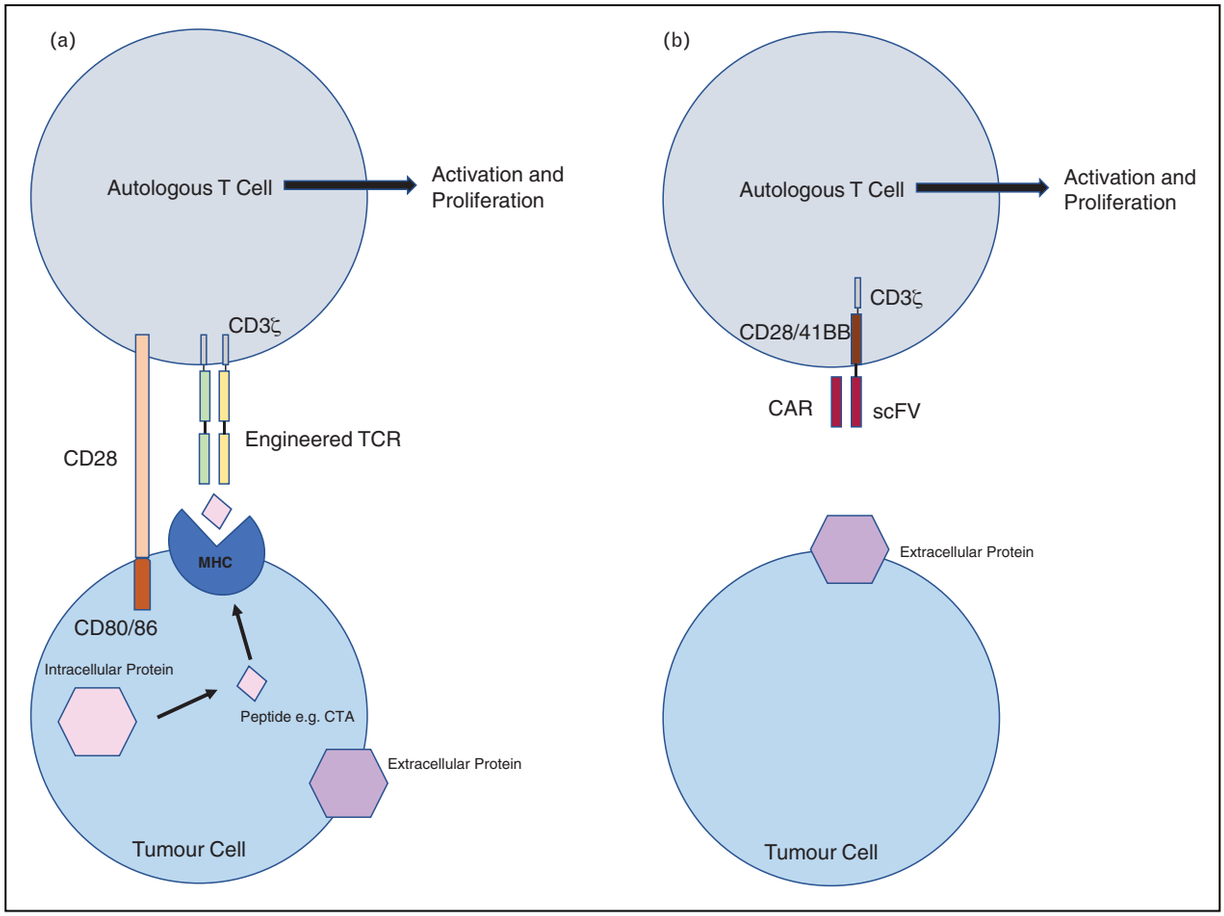

FIGURE 1. (a) T-cell receptor-transduced T cell. The high affinity T-cell receptor can recognize a specific intracellular peptide presented on cell surface histocompatibility complex molecules. (b) Chimeric antigen receptor T cell. Chimeric antigen receptors comprise an antigen-binding, extracellular domain coupled to the intracellular signaling CD3ζ domain of a T-cell receptor, in addition to costimulatory receptors. They can recognize extracellular proteins only and are thus independent of HLA type.

persistence of anti-NY-ESO-1-specific T cells at 4 weeks was not associated with response.

Autologous T cells transduced with another anti-NY-ESO-1 TCR, NY-ESO-1^{c259}, were evaluated in a synovial sarcoma trial, where confirmed anti-tumor responses over several months occurred in 50% of 12 patients. Regenerative pools of NY-ESO-1^{c259} T cells persisted for at least 6 months, providing an ongoing supply of effectors cells thought to underlie sustained responses [71]. A basket phase Ib trial of TCR TBI-1301, which included four patients with synovial sarcoma, concluded a best response of partial responses and no dose-limiting toxicities [72▪▪].

CTA-targeted vaccines stimulate dendritic cells and induce NY-ESO-1 presentation and a subsequent T-cell mediated immune response, for example LV305 and its later prime-boosted iteration CMB305 (vaccine and NY-ESO-1 recombinant protein and GLA-SE, a TLR-4 agonist). A phase Ib trial of CMB305, in which 81% of patients had sarcomas, concluded a disease control rate of 61.9% and observed anti-NY-ESO-1 antibody and T-cell responses in 62.9 and 47.4% of cases [73]. In a randomized phase II trial, there was no difference in median progression free survival and OS between combination CMB305 and atezolizumab vs. atezolizumab alone [28].

Endogenous NY-ESO-1 specific T cells have been safely transferred in a phase I trial; however, all patients experienced disease progression and transferred cells lacked markers of proliferation or activation [30,74▪]. Proliferation was induced when the T cells were cultured *ex vivo* with IL-15, supporting the evaluation of this cytokine for use following cell infusion to support responses.

The MAGE proteins are a family of CTAs consisting of MAGE-A, MAGE-B and MAGE-C, with associated subfamilies. MAGE-A1 was the first identified tumor T-cell antigen [75]. The MAGE-A family

have been implicated in the inhibition of p53 and its tumor suppressor properties, both by direct binding and leading to raised levels of the p53 inhibitor murine double minute 4 in cancer cells [64,65]. TCRs that target MAGE-A4, MAGE-A3 and MAGE-A10 are under clinical evaluation (Table 2). MAGE-A4 is expressed in high levels in synovial sarcoma, and expression usually occurs alongside NY-ESO-1 [76,77].

ADP-A2M4 is a developmental ACT that recognizes the HLA-A2-restricted MAGE-A4 peptide GVYDGREHTV, and demonstrated antitumor efficacy when tested in both in-vitro cells lines and in-vivo xenografted murine melanoma models [78]. A basket phase I trial (NCT03132922) of ADP-A2M4 SPEAR T cells (containing the MAGE-A4^{c1032} TCR) reported seven partial responses in synovial sarcoma, overall response rate of 44% with durable responses lasting up to 6 months. This led to a phase II trial in synovial sarcoma and MRCL (SPEARHEAD-1; NCT04044768), and a phase I basket trial of a next-generation SPEAR T-cell targeting MAGE-A4 (SPEARHEAD-1; NCT04044859) [79▪▪,80▪▪]. Levels of MAGE-A4 expression correlated with response, and response was dose-dependent [80▪▪].

The evaluation of MAGE-A3-specific TCRs has presented more challenges; a clinically meaningful response in synovial sarcoma was noted in a phase I trial of an autologous HLA-A∗0201-restricted, MAGE-A3/9-specific TCR, but cross-reactivity with an HLA-A∗0201-restricted MAGE-A12 epitope present in brain tissue led to severe neurotoxicity, observed in three of nine patients in total (lethal in two) [81]. In a similar trial, a MAGE-A3-directed TCR, recognition of titin protein in heart muscle led to lethal toxicity [82,83]. In a more recent basket trial, MAGE-A3-specific CD4^{+} T cells were safely transferred to 17 patients, where one osteosarcoma patient had a partial response (4 months duration), but no response in synovial sarcoma [84].

PRAME is a CTA that contributes to cancer cell survival by inhibiting apoptosis, proliferation arrest and inhibiting retinoic acid receptor signaling. High, homogenous expression of PRAME of up to 100% has been described in synovial sarcoma, though there is questionable reliability of available assays [76,85–87]. PRAME expression and coexpression alongside NY-ESO-1 and MAGE-A4 may be associated with adverse prognosis in synovial sarcoma, and its expression may be negatively correlated with MHC class I presentation, limiting its use as a target for HLA-restricted ACT [76,88]. Clinical trials evaluating PRAME-directed TCRs in solid cancers are ongoing (NCT04262466; NCT03686124). Phase Ia results for NCT03686124 evaluating IMA203, demonstrated all 12 evaluable patients achieved disease control and three synovial sarcoma patients had partial responses.

Recognizing that expression of NY-ESO-1, the MAGE family and PRAME antigens often coexist in STS, there are active clinical trials of endogenous T-cell therapy exploiting multiantigen targets (NCT01477021, NCT02239861) [30].

CHALLENGES AND FUTURE DIRECTIONS

Specialist infrastructure required for leukapheresis, product manufacture, lymphodepletion and toxicity management are important considerations with regard to modified T-cell therapy and will likely limit use to dedicated centers. Data on efficacy, durability of disease control and applicability to a small proportion of cancer patients must be viewed in the context of labor intensive and expensive product preparation and administration.

Restriction to patients who are HLA A∗02:0-positive poses another significant limitation, as this HLA type is most commonly observed in Caucasian populations (50%), with lower expression levels in Asian and African populations [89]. Long lag times of several weeks from patient identification to product delivery exclude patients with rapidly progressive disease. CAR-T cells directed against NY-ESO-1 have shown efficacy in murine models of NY-ESO-1-positive myeloma, supporting their exploration in sarcomas where modified TCRs have shown success, overcoming HLA restriction [90].

CRISPR–Cas9 editing to delete genes encoding endogenous TCR chains enhances expression of engineered NY-ESO-1-TCRs. This method was utilized in a phase I trial which included 1 sarcoma patient with durable stable disease and is an example of efforts to increase on-target specificity and durability of treatment responses [91,92]. A gene encoding PD-1 was also removed to enhance the antitumor response. Additionally, the application of stimulatory cytokines to support modified T-cell persistence, proliferation and activity may alter the immunological phenotype of synovial sarcoma and MRCL, specifically the challenges presented by low T-cell infiltration and associated reduced HLA/MHC expression [8,58,93]. Coadministration of IFNγ, while inducing MHC 1 expression, T-cell infiltration and PD-L1 expression [31], resulted in fatal myocarditis when included in the conditioning regime for ACT, and investigators concluded that this should not be coadministered with high dose alkylating agents or IL-2 [94]. IFNα has been safely utilized in TIL therapy, and IFNγ may indeed have its uses in sensitizing patients to ACT [95], but should be evaluated in the medium – to long term after cell infusion, for example several weeks after or

at the time of progression. Ex-vivo application of IL-15 has stimulated activation and proliferation of persisting NY-ESO-1 endogenous T cells supporting its clinical evaluation as a sensitizing agent [74▪]. Importantly, high dose IL-2 was utilized following cell infusion in early trials of NY-ESO-1-directed TCRs and warrants further consideration once the safety and activity of this approach has been reliably demonstrated [69,70]. While alone likely lacking the capacity to enhance the endogenous immune response to antitumor effect in nonimmunogenic sarcomas, checkpoint blockade may play a role in optimizing proliferation and persistence of adoptively transferred antigen-specific cells and/or impact the TME. It also needs to be acknowledged that adoptively transferred cells have a limited lifespan. To promote durable disease control, there is likely a requirement for ACT-induced epitope spreading and endogenous immunity; translational efforts to characterize and identify how to promote such endogenous responses will be important going forwards.

There is a continued lack of consensus on an optimal lymphodepletion regime for ACT with TCR-transduced T cells. Current regimes have evolved following their use in TIL melanoma trials, and from CD19-targeted CAR-T cell therapy [34,74▪,96,97]. Lymphodepletion augments the antitumor effect of transferred cells by eliminating endogenous suppressor T-cell populations and competition for cytokines including IL-7 and IL-15. Fludarabine in particular has been demonstrated to have a significant impact on IL-7 and IL-15-mediated endogenous T-cell responses [34,71]. Notably, expansion cohort evaluation of NY-ESO-1^{c259} in synovial sarcoma patients with less intensive lymphodepletion, concluded response rates of 30% compared with 50% in the initial cohort [98]. Translational evaluation in the same study confirmed the necessity of fludarabine preconditioning to elicit elevated post-infusion levels of IL-7 and IL-15, and that sole use of cyclophosphamide is not sufficient. Preparative total body irradiation alongside lymphodepletion does not appear associated with improved response to ACT [99]. Both synovial sarcoma and MRCL demonstrate sensitivity to alkylating agents, utilized in conventional treatment paradigms in combination with doxorubicin or as single agent in anthracycline-refractory disease. It is therefore necessary to accept that responses to modified T-cell therapy may in part be contributed to by the use of cyclophosphamide [34,100]. The toxicity profile of modified T-cell therapy is conferred both by lymphodepletion and resultant pancytopenia alongside immune-mediated toxicity following cell infusion, specifically the risk of cytokine release syndrome, which can result in rapid and severe organ failure. Importantly, unlike with CD19-targeted CAR-T-cell therapy in hematological malignancies, immune effector cell-associated neurotoxicity syndrome has not been reported with anti-CTA-directed TCR therapy, which overall is well tolerated [48,69,71]. Two patients treated with anti-NY-ESO-1 TCRs have developed Guillain–Barre syndrome and vigilance for a potentially diverse range of immune and virus-mediated toxicities is required [101]. Normal tissue toxicities are expected to be limited in CTA-directed therapy, as these antigens are by their nature absent outside of malignant tissue.

CONCLUSION

Modified T-cell therapy offers an opportunity in sarcomas where reliably expressed, tumor-specific target antigens can be identified, especially where the TME is not well suited to checkpoint inhibition or TILs. While having the advantage of circumventing HLA-restriction, the use of CARs in sarcomas may be limited by the requirement for extracellular targets. Engineered T cells modified to express CTA-specific TCRs, most notably targeting NY-ESO-1 and MAGE-A4, have shown promise in synovial sarcoma/MRCL. HLA-restriction and limitations of the sarcoma TME are challenges affecting clinical applicability, and tools to improve the specificity of TCRs and augment responses are necessary to harness the full potential of this approach.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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