Immunotherapy in Sarcoma
Where Do Things Stand?

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KEYWORDS

• Soft tissue sarcoma • Immune checkpoint inhibitors • Tumor microenvironment • Tertiary lymphoid structure • Adoptive cellular therapy • Immunotherapy

KEY POINTS

- ICIs induce responses in only about 20% of unselected sarcoma patients in clinical trials.
- Efficacy signals with checkpoint blockade may be higher in alveolar soft part sarcoma , angiosarcoma, , and dLPS.
- Current trials are exploring combination therapies with checkpoint blockade to overcome immune evasion mechanisms.
- Adoptive cellular therapies are promising, but studies have been limited to SS and myxoid/round cell liposarcomas.
- Biomarkers of efficacy are under investigation and critical to improve the selection of patients for immunotherapy clinical trials.

INTRODUCTION

The development of modern immunotherapy, including immune checkpoint inhibitors (ICIs) that block PD1/PD-L1 and CTLA-4, and adoptive cellular therapies, has created an entirely new paradigm for cancer treatment, with remarkable activity in many different solid and hematologic malignancies. Sarcomas, a rare and heterogeneous group of over 150 different bone and soft tissue cancers, have long been theorized to be susceptible to immune recognition and attack. With this explosion of therapeutic opportunities, the past 5 years have seen remarkable growth in clinical trials and laboratory efforts to explore immunotherapy for bone and soft tissue sarcomas (STSs).

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However, early experiences with ICIs have been disappointing in trials of unselected sarcoma subtypes, with collective responses of only about 20%. Although adoptive cellular therapies targeting cancer testis antigens (CTAs) such as NY-ESO-1 and MAGE-A4 are highly promising, these strategies are limited by human leukocyte antigen (HLA) allele frequency in the general population, and only two sarcoma subtypes reliably express these targets. The majority of sarcomas are immunologically "cold" with sparse immune infiltration, which may explain the poor response to immunotherapy. The lack of immune responses may hinge on the genetic background, with sarcomas often having low tumor mutational burden (TMB) or being driven by translocations, which may limit neoantigens for exploitation of immune responses. Finally, the small sample sizes of clinical trials, and the heterogeneity of biomarker explorations in trials or in laboratory settings challenges our ability to select optimal patients for future immunotherapy clinical trials. In this review, we will discuss the current state of immunotherapy for sarcomas, highlighting notable prior investigations of immunotherapy, reviewing ongoing clinical trials, and speculating on future directions for the field.

IMMUNE CHECKPOINT INHIBITORS

Immune checkpoint proteins serve as critical regulators of immune responses, and blocking antibodies to the PD1/PD-L1 and CTLA-4 inhibitory axes are now used as monotherapy or in combinations with chemotherapies in the first or second line in more than 50 cancer types.[1] The earliest trial of ipilimumab monotherapy in synovial sarcoma (SS) patients was terminated early due to lack of response.[2] The pivotal phase 2 trial of pembrolizumab in bone and STSs showed responses in 4 of 10 patients with undifferentiated pleomorphic sarcoma (UPS) and 2 of 10 patients with dedifferentiated liposarcoma (dLPS).[3] Minimal activity was seen in SS, leiomyosarcoma (LMS), or bone sarcomas. Shortly afterward, a phase II trial of nivolumab versus ipilimumab with nivolumab in bone sarcomas and STS confirmed low responses with nivolumab alone, but 6 of 38 patients treated with combination ipilimumab/nivolumab achieved a response, including two complete responses in myxofibrosarcoma (MFS) and uterine leiomyosarcoma (uLMS).[4] Subsequent expansion cohorts in both the pembrolizumab monotherapy and ipilimumab/nivolumab studies further explored activity in UPS and dLPS, with response rates falling to approximately 23% for UPS, and 10% overall for dLPS. The combination of nivolumab with ipilimumab led to an overall response rate (ORR) of 17% and 29% in dLPS and UPS, respectively.[5,6] Additional studies have identified strong signals of activity for alveolar soft part sarcoma (ASPS) and cutaneous angiosarcomas. More than 150 patients with ASPS have been treated in clinical trials including PD1/PD-L1 antibodies, with responses ranging from 7.1% to more than 50% (**Table 1**). For angiosarcomas, multiple retrospective case reports[7–9] and genetic profiling of patients identifying frequent UV damage signatures in cutaneous subtypes[10] formed the basis for an expansion cohort in the dual anti-CTLA-4 and anti-PD1 blockade in rare tumor (DART) study run through SWOG.[11] Of 16 evaluable patients, the ORR was 25%; however, three of five patients with primary cutaneous scalp/face angiosarcoma attained a confirmed response, with 6-month progression-free survival (PFS) rate of 38%.

Apart from the activity in ASPS, angiosarcoma, UPS and dLPS, and the occasional sporadic responses in other sarcoma types, the overall modest responses with ICI monotherapy suggest that other resistance mechanisms may be limiting the efficacy of checkpoint blockade, potentially through a suppressive immune microenvironment. Thus, combination strategies with various chemotherapies and targeted therapies are

Table 1
Responses of alveolar soft part sarcoma to regimens containing immune checkpoint inhibitors

Therapy	N	Response Rate (95% CI)	mPFS (months, 95% CI)	Reference
Nivolumab (OSCAR trial)	14	7.1% (0.2–33.9)	6.0 (3.7–9.3)	Kawai et al,[87] CTOS 2020
Retrospective multi-institutional series (monotherapy, N = 31, combination N = 29)	60	40.4% NR	13.4 (10.1–16.7)	Hindi et al,[88] ASCO 2021
Durvalumab/Tremelimumab (ASPS subset)	10	50% NR	34.23 (1.84 – NR)	Somaiah et al,[89] ASCO 2020
Atezolizumab	44	37.2% NR	NR	Naqash et al,[90] ASCO 2021
Axitinib/pembrolizumab	11	54.5% (24.6–81.9)	12.4 (2.7–22.3)	Wilky et al,[14] 2019
Geptanolimab	37	37.8% (22.5–55.2)	6.9 (5.0 – NR)	Shi et al,[91] 2020
Toripalimab, ASPS subset	12	25.0%	11.1 (NR)	Yang et al,[92] 2020

Abbreviations: ASCO, American Society of Clinical Oncology; ASPS, alveolar soft part sarcoma; CTOS, Connective Tissue Oncology Society; mPFS, median progression-free survival; NR, not reached, not reported.

increasingly being explored (**Table 2**). The next series of ICI trials for sarcomas were aimed at suppressive immune phenotypes such as T-regulatory cells or tumor-associated macrophages (TAMs) or suppressive cytokines such as vascular endothelial growth factor (VEGF). The PEMBROSARC study combined metronomic cyclophosphamide, which has been shown to suppress T-regulatory cells and augment T cell and natural killer (NK) cell function, with pembrolizumab in bone and STSs.[12] Unfortunately, only 1 of 50 STS patients achieved a response, and only three were progression free at 6 months. A later cohort of 17 osteosarcoma patients treated in this study revealed one patient achieving a response with three others experiencing tumor shrinkage; however, the median PFS was still low at only 1.4 months.[13] Additional studies have investigated tyrosine kinase inhibitors (TKIs) along with PD1 blockade. In a Phase 2 study of the pan VEGFR inhibitor axitinib with pembrolizumab, remarkable responses were observed in 6 of 11 patients with ASPS; however, only two responses in epithelioid sarcoma and soft tissue LMS were observed among the other STS patients on the study.[14] A phase 2 trial of the broader spectrum TKI sunitinib with nivolumab was also completed.[15] Of 58 evaluable patients, the ORR was 21%, with responses observed in angiosarcoma, clear cell sarcoma, ASPS, extraskeletal myxoid chondrosarcoma, and SS. The 6-month PFS rate was 48% by central assessment. Finally, a third study combined the selective VEGFR-2 TKI apatinib with the anti-PD1 antibody camrelizumab for 43 patients with osteosarcomas.[16] The ORR was 20.9%, with two long-term responders. The 6-month PFS rate was 50.9%. Ongoing studies are continuing to explore immunotherapy combinations using TKIs impacting broader kinomes and proven activity in sarcoma, such as cabozantinib (NCT04339738, angiosarcoma; NCT04551430, STS; NCT05019703, osteosarcoma). However, given the limited responses for all-comers, additional biomarkers are needed to identify the subset of patients likely to benefit from this approach.

Emerging transcriptomic data have shed light on immune classifications of sarcomas, identifying an immune-high subset that correlates with response to pembrolizumab monotherapy, and a vascular-enriched subset that has not yet been correlated with responses to TKI-containing combinations.[17] The majority of sarcomas have very

Table 2
Summary of responses to checkpoint inhibitors and combinations in various sarcoma subtypes

Sarcoma Subtypes	Checkpoint Inhibitor	Combination Partner	N	ORR	Median PFS (months)	Reference
LMS, UPS, GIST, others	Pembrolizumab	Cyclophosphamide	57	2%	1.4	Toulmonde et al[13]
STS	Pembrolizumab	Axitinib	33	25%	4.7	Wilky et al,[14] 2019
All Sarcoma	Nivolumab ± ipilimumab	None	43/42	5%/16%	1.7/4.1	D'Angelo et al,[4] 2018
All Sarcoma	Pembrolizumab	None	84	18%/5%	4.5/2	Tawbi et al,[3] 2017
STS	Ipilimumab	Dasatinib	28	0%	2.8	D'Angelo et al,[93] 2017
All Sarcoma	Durvalumab	Tremelimumab	57	14.3%	4.5	Somaiah et al,[89] 2020
GIST, UPS, DDLPS	Nivolumab ± ipilimumab	None	66	0% −14%	1.5–5.5	Chen et al,[6] 2020
STS	Pembrolizumab	Doxorubicin	30	33%	6.9	Livingston et al,[20] 2021
STS	Ipilimumab/Nivolumab	Trabectedin	41	19.50%	6	Gordon et al,[94] 2019
STS	Nivolumab	Sunitinib	68	13%	5.6	Martin-Broto et al,[15] 2020
Bone	Nivolumab	Sunitinib	40	5%	3.7	Palmerini et al,[95] 2020
All Sarcomas	Pembrolizumab	Doxorubicin	37	22%	8.1	Pollack et al,[21] 2020

low expression of immune-related genes and suggest a failure to mount an immune response due to innate lack of immunogenicity, either from lack of neoantigens or failure of antigen presentation and recognition. Thus, the newest wave of combination ICI clinical trials for sarcomas is now focused on inducing immunogenicity that can then be perpetuated by downstream checkpoint blockade. The main strategies being explored include radiation therapy, cytotoxic chemotherapy, and other agents such as cytokines that aim to generate novel neoantigens or induce the production of danger signals from dying or injured tumor cells to draw in innate immune cells.

Radiation has been shown in numerous cancers to increase immunogenic cell death, boost antigen-presenting cell priming, and activate effector T cell responses through the formation of double-stranded DNA breaks that can activate the cGAS-STING pathway and promote inflammatory cytokine production.[18] SU2C-SARC032 is a randomized Phase 2 trial of 105 patients with high-grade stage III extremity UPS or dLPS treated with preoperative radiation therapy with or without adjuvant pembrolizumab (NCT02301039). With accrual completed, the results of this study may greatly impact the upfront management of sarcomas, leading to potential improvement in distant metastasis and pathologic response.

Cytotoxic chemotherapy, particularly doxorubicin and other anthracyclines, has been shown in a variety of cancers to induce the release of damage-associated markers and cytokines and promote type 1 interferon (IFN) production by tumor cells to improve immunogenicity.[19] Two single-arm phase 2 studies have recently been reported showing promising activity of doxorubicin with pembrolizumab for advanced/metastatic sarcomas.[20,21] The ORR was 19% in the Pollack study, which included bone and STS, and 36.7% in the Livingston study, which was all STS. Pollack reported a median PFS of 8.1 months with a 24-week PFS rate of 73%. The heterogeneous study population including a fair number of atypical sarcoma subtypes may have influenced the longer PFS; however, a subset of patients showed prolonged benefit. The median PFS in the Livingston study was 5.7 months, and 6-month PFS rate was 44% in a more selected STS population. Overall, the combination of doxorubicin with immune therapy has a solid rationale, and multiple ongoing trials are seeking to improve these outcomes by incorporating ifosfamide (NCT04356872, NCT04606108) or combination CTLA-4/PD1 blockade (NCT04028063). Other cytotoxics also have profound impacts on immune reactivity and are being explored in combination studies with ICIs, including gemcitabine (NCT04577014, NCT03123276, NCT04535713), trabectedin (NCT03138161), and eribulin (NCT03899805).

Another interesting strategy was explored in a phase 2 study combining an IL-2 pathway agonist, NKTR-214, with nivolumab for bone and STS (NCT03282344). Although some significant and durable responses were observed, unraveling the contribution of NKTR-214 to PD-1 blockade is difficult, an issue with all single-arm combination studies. Full results and correlative data from this study are upcoming and may shed light on underlying mechanisms of response and resistance. Similarly, Pollack and colleagues are conducting a Phase 2 trial of IFN-γ with pembrolizumab (NCT03063632) based on prior laboratory data showing that IFN-γ could upregulate MHC Class 1 expression and subsequent T-cell infiltration.[22] Thus, these are examples of directly targeting key cytokines instrumental to the early immune response and hopefully overcoming the innate resistance seen in so many sarcomas.

Biomarkers of Efficacy

As the results of these ongoing studies emerge, and critical correlative data from previously completed studies are released, we may have a better sense of biomarkers that can correlate with responses to various combinations to build into future trials.

To date, many investigations have queried sarcoma tissue archives to determine whether biomarkers that have predicted responses to checkpoint inhibitors in other cancers hold true in sarcomas. Overall, the results of these studies have been conflicting, confounded by histologic subtype differences, and limitations of assays used. Additionally, with many of these studies performed on small biopsies, sampling bias plays a real role, considering that many immune cells may lay on the leading edge of tumors or excluded outside the tumor in the stroma.

Tumor and tumor-infiltrating lymphocyte (TIL) PD1 and PD-L1 expression have been a reliable indicator of an established but exhausted immune response that can be rejuvenated with checkpoint blockade in other types of cancers.[23] In sarcomas, various studies have explored PD1/PD-L1 expression and how it may affect overall survival (OS) and event-free survival (EFS). Some studies suggest that elevated PD1/PD-L1 expression is associated with worse overall survival; however, others suggest it is favorable. Further confounding these results is that some studies use protein expression with various antibody clones, whereas others report genetic expression that are not interchangable. A recent comprehensive meta-analysis containing 15 independent studies and 1,451 patients showed that high PD-L1 expression was associated with worse overall survival (HR 1.27, $P = .000$) and worse EFS (HR 2.05, $P = .000$).[24] However, these retrospective analyses are not correlated with clinical outcomes and do not take into account the recent use of immunotherapy. Interestingly, correlative studies exploring PD-L1 expression in sarcomas treated with ICIs are similarly contradictory. Although the numbers of patients are small, PD-L1 tumor expression does not appear to be required for response to therapy, although responders often exhibit PD-L1 expression. A recent review of 154 patients treated on checkpoint inhibitor trials with PD-L1 expression status showed 6/20 PD-L1-positive patients achieved response (30%); however, 9 of 133 PD-L1 negative patients also showed a response to treatment.[25]

Another major biomarker that remains underexplored in sarcomas is the presence of TILs. Similarly to PD-L1, the presence of TILs has been shown to have either negative or positive prognostic significance in reported clinical trials reviewed in a study.[26] However, many of these studies do not go on to further profile these cells, and heterozygous TIL populations could include activated or suppressive T cells, including $CD8^+$, $CD4^+$, or T-regulatory cells, NK cells, B cells, or myeloid/macrophage cells, all with different functions and significance. Although numerous studies have retrospectively reported on the prevalence of these phenotypes and associations with OS, EFS, or metastasis-free survival in various sarcoma subtypes,[26] there is very limited data on associations with ICI response. Responding patients with pembrolizumab monotherapy had significantly higher CD8+ T-cell infiltration and PD-L1+ TAMs at baseline compared with nonresponders.[27] More recently, work from Petitprez and colleagues demonstrated that the presence of tertiary lymphoid structures (TLSs) containing DC-$LAMP^+$ dendritic cells and $CD20^+$ B cell aggregates in sarcomas also correlated retrospectively with response to pembrolizumab.[17] Building on these observations, an extended cohort of the PEMBROSARC trial enrolled 48 TLS positive sarcoma patients from 240 screened (20%) who were eligible to receive pembrolizumab and oral cyclophosphamide.[28] Among the 35 evaluable patients, 30% displayed objective response, 33.3% had stable disease, and PFS and OS were 4.1 and 14.5 months, respectively. These promising results contrast with the initial ORR of 2% and PFS of 1.4 months reported in the unselected PEMBROSARC population.[12] Overall, further investigation into the impact of TILs is desperately needed to further refine the selection of patients for combination studies that potentially target these other phenotypes.

A promising strategy for characterizing sarcomas and potentially predicting responses to ICI has proven to be advance in sequencing technology. TMB and

microsatellite instability (MSI) are used as biomarkers to predict response to immunotherapy in various cancers. Sarcomas generally have low TMB, with an average of 1.06 mutations/Mb reported in TCGA analysis.[29] However, hypermutated sarcomas with high levels of UV-associated mutations have recently been identified, mainly angiosarcoma (especially stemmed from face and scalp) and MPNST.[30] A comprehensive analysis of 47 angiosarcomas prospectively registered in a cohort reported a median TMB of 3.3 mutations/Mb in the full cohort, and a median TMB in the face and scalp angiosarcoma of 20.7 mutations/Mb, significantly higher than in all other angiosarcoma subclassifications (2.8 mutations/Mb; p 1.1 10^{-5}). Among 10 patients with face and scalp angiosarcoma, two patients were treated with ICI and showed exceptional and durable responses. No clinical benefit was observed in the 3 of 26 patients with angiosarcoma with other localizations (outside face and scalp) treated with anti-PD1.[10] TMB was also demonstrated to correlate with mismatch repair-deficiency (MMR-D) in a series of 304 STS.[31] A low proportion of sarcomas (7/304%, 2.3%) was classified as MMR-D. MMR-D sarcomas showed a median TMB significantly higher than MMR-proficient sarcomas (16 vs 4.6, P <.001). Results from larger sarcoma cohorts are expected to determine whether TMB may be used as a single biomarker to predict a benefit to immunotherapy or whether the global context (MMR status, tumor type, carcinogen exposure) should be required. MSI-high signature in a tissue agnostic fashion led to the approval of pembrolizumab by FDA. MSI status in sarcomas was assessed across 71 samples of various STS and remains an uncommon event.[32]

In addition to identifying the rarer cases of STS that exhibit high TMB or MSI high status, recent studies have shown that bulk transcriptomic data can be deconvoluted to extract contributions of various immune cell signatures. These techniques have allowed for the clustering of various sarcomas into immune low, moderate, or high activity signatures. Multiple studies mining available transcriptomic data have created sarcoma immune subsets; however, only the Petitprez study provides the correlation with responses to PD1 monotherapy.[17,33,34] Patients in the high immune expression SIC-E were more likely to achieve an objective response and to have a favorable PFS.

Overall, given the complexity of the immune microenvironment in sarcomas, including intertumor and intratumor heterogeneity, developing biomarkers for ICIs remains a critical need and an area of active exploration.

ADOPTIVE CELLULAR THERAPIES

As we have discussed, one of the fundamental immune evasion mechanisms in sarcomas may be the inability to mount an immune response due to poor neoantigens or faulty antigen recognition, leading to a failure to generate an adequate supply of tumor-specific T cells. Adoptive cellular therapies aim to bypass this step, by providing a large volume of autologous T cells that are either collected from the primary tumor or collected from peripheral blood and engineered to be specific for a particular antigen and expanded. Most products require lymphodepleting chemotherapy before administration. Adoptive cellular products can include engineered T-cell receptor (TCR), chimeric antigen receptor (CAR) T-cell therapies, TILs, and NK cells.

Engineered T-Cell Receptor Therapy Targeting Cancer Testis Antigens

New York esophageal squamous cell carcinoma 1 (NY-ESO-1) is a CTA, a protein involved in immunologic maturation that is typically restricted to human male germ cells, that exhibited increased expression in sarcomas, primarily SS and myxoid/round

cell liposarcoma.[35,36] Because NY-ESO-1 is an intracellular antigen, which must be processed and presented in association with MHC, these targets are better suited for engineered TCR T cells. Compared with CAR-T, engineered TCR T cells require matched HLA allele subtypes in patients, generally HLA-A*02, which is found in roughly 30% of the population, and can be plagued with greater off-tumor target toxicity compared with CAR T cells.[37] In a Phase 1 clinical trial of NY-ESO-1 TCR T cell therapy that included 10 SS patients with HLA-A*02 positive tumors, no adverse fatal events occurred with persistence of T cells *in vivo*.[38] A Phase 2 clinical trial has recently begun to assess overall response, response duration, PFS, OS, safety, and tolerability.[39–42]

The melanoma antigen gene (MAGE) protein family is a highly conserved group of proteins that are present on the X chromosome and in reproductive tissues. However, MAGE-A4 has been found to be broadly expressed in many tumor types, including several reports showing expression of both NY-ESO-1 and MAGE-A4 in STS, especially SS where 70.6% are positive for either marker.[43,44] A Phase 2 study of 35 patients with SS who had been treated with a TCR T cell, afami-cel, showed showed a favorable safety profile with complete and durable responses in most of the patients.[45]

CAR T-Cell Therapies

CAR T-cell therapies, which combine the targeted specificity of antibodies with the effective capabilities of T cells, offer a promising therapeutic intervention based on their successes in treating CD19+ acute lymphoblastic leukemia and B-cell lymphomas. There are several generations of CAR T cells that differ based on their intracellular costimulatory domains, such as 4-1BB, CD28, and OX40, which enhance proliferation and survival.[46] Patients undergoing CAR T-cell therapy experience an increased degree of immune stimulation and inflammation resulting in systemic cytokine release syndrome (CRS) in some cases.[47] However, this complication is well managed with IL-6 inhibitors, like Tocilizumab, and steroids for neurotoxicity. Thus far, CAR-T protocols have struggled in solid tumors, mainly due to difficulties in finding conserved targets without prohibitive toxicity to normal organs carrying the same antigens.

For sarcomas, multiple targets have been explored in prior and ongoing clinical trials. Human epidermal growth factor receptor 2 (HER2) is a ligand that can activate downstream pathways of Ras/Raf/MEK/ERK1/2 and phospholipases to promote oncogenesis.[48] In a Phase I/II clinical trial in 19 patients with $HER2^{+}$ osteosarcomas, Ewing sarcoma, neuroectodermal tumor, and desmoplastic small round cell tumor, HER2-CAR T-cell therapy demonstrated no adverse side effects with four patients achieving stable disease for 12 weeks to 14 months.[49] The study showed that the median overall survival was 10.3 months with a median follow-up time of 10.1 months. A Phase I study in 10 patients with refractory/metastatic HER2+ sarcoma showed that lymphodepletion chemotherapy followed by autologous HER2-CAR T cell therapy was associated with improved clinical benefit.[50] Results showed that one patient with osteosarcoma achieved complete response and two others had stable disease. In patients with rhabdomyosarcoma, two achieved complete response and the third exhibited stable disease. The patient with Ewing sarcoma also aachievechieved achieved stable disease. Although this therapy shows promise in patients with HER2+ sarcomas, additional studies are combining CAR T cells with ICIs (NCT04995003) to improve efficacy. Phase 1 clinical trials are currently ongoing using EGFR (NCT03618381) and GD2 (NCT02107963, NCT04539366, NCT03721068, NCT03635632) CAR T cells for pediatric sarcomas, including osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma expressing these surface markers.

Other targets in earlier phases of development include insulin-like growth factor 1 receptor (IGF-1R), a transmembrane receptor tyrosine kinase, and promoter of tumor cell survival, which has has demonstrated prognostic significance in sarcomas where several cell lines have been sensitive to IGF-1R inhibition.[51] Tyrosine kinase orphan-like receptor 1 (ROR1), a transmembrane protein involved in cancer cell migration, invasion, and metastasis, is is overexpressed in osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma.[52] IGF-1R and ROR-targeted CAR T-cell therapies are still in early stages in humans.[53] CD44v6, a cancer cell marker of metastasis and tumor progression, is associated with poor prognosis in osteosarcoma patients.[54,55] It is expressed in 40% of STSs, including fibrosarcoma, LMS, liposarcoma, and UPS. When used as a therapeutic target, CAR-redirected cytokine-induced killer (CIK) T cells exhibited greater tumor growth delay compared with untreated and control-treated mouse cohorts.[56] Thus, CD44v6 remains a promising target for future study. Finally, NK cell activating receptor group 2-member D ligand (NKG2DL), a ligand from the NKG2D family involved in the activation of macrophages, T cells, and NK cells to promote antitumor immunity, is rarely expressed in normal tissue but overexpressed in osteosarcoma and Ewing's sarcoma.[57,58] Second-generation NKG2D-directed CAR-T cells against osteosarcoma and Ewing's sarcoma demonstrated increased cytotoxicity, lower tumor burden, and increased overall survival in murine models.[58,59]

TIL Therapies

TILs, which demonstrate antitumor activity *in vivo*, are extracted from resected or biopsied human tumors and undergo *ex vivo* expansion to be administered to patients following a lymphodepletion regimen.[60,61] Although they have demonstrated therapeutic efficacy in melanoma of at least 50%, their growth from other solid tumors has been varied.[62–64] The limited ability to expand them presents a challenge to their global application to serve large numbers of patients with cancer. Due to its personalized nature, each patient requires a unique infusion product to be produced, which will largely drive up costs.[65] Although robust and reproducible, the largest toxicities associated with TIL therapy include the lymphodepleting regimens, the use of interleukin-2 (IL-2), and the associated toxicity.[66–68] Recent work from Mullinax and colleagues has shown feasibility in establishing TIL cultures from sarcoma resections with about 25% of resected specimens yielding sufficient TILs for a clinical product ($\geq 2 \times 10^7$ cells).[69] Clinical trials using TIL technology have been ongoing through the NCI and other institutions, with Mullinax and colleagues currently conducting a dedicated Phase 1 clinical trial (NCT04052334). Phase 2 clinical trials studying the efficacy of TIL therapy are also enrolling for recurrent ovarian carcinosarcoma (NCT03610490) and STSs (NCT03935893).

Natural Killer Cell Therapies

NK cells, members of the innate lymphoid cell family, are effective defenders against cells infected with pathogens and tumors through their ability to express a diverse array of surface receptors.[70] Because they lack MHCs and possess cancer cell recognition capability, many studies are currently being carried out to use NK cells as novel therapeutic tools. However, despite their promise, they are limited by tumor immunoevasion, an inhospitable tumor microenvironment, and inadequate homing properties.[71,72] Irrespective, research is currently being performed to circumvent these limitations and unlock the potential of this therapy (NCT02890758, NCT02409576, NCT01875601, NCT03420963).[73]

Future Directions

Adoptive cellular therapies offer promising personalized therapies but are still in their infancy for solid tumors. Despite the present limitations for TILs, NK cells, and CAR T cells, they have all undergone refinements to improve efficacy and decrease toxicity. Methods are currently being investigated to enrich TILs through selection for CD137 or PD-1 in hopes of increasing their antitumor activity.[74,75] NK cells offer versatile potential, but further studies are needed to better understand their mechanisms of action, activation, and suppression within the tumor microenvironment.[76] New studies are attempting to augment NK cell anticancer properties through genetic modification and altered priming strategies to enhance cancer recognition, improve tumor homing, and reduce resistance.[77] The success of CAR T-cell therapies in hematological malignancies has expanded their utility to solid tumors, including sarcomas. Although CARs have demonstrated efficacy in *in vitro* models their biggest test will be their long-term efficacy in clinical trials. As more potent tumor-specific targets are defined, CAR T-cell constructs will be modified with new targets added to improve their efficacy especially in the solid tumor microenvironment.[78] Next-generation therapies should be more robust, safer, and better equipped to overcome the immunosuppressive microenvironment.

NOVEL THERAPIES

Talimogene laherparepvec (T-VEC) showed increased tumor-specific immune activation via augmenting antigen presentation and T-cell priming. A phase II clinical trial assessed the efficacy of the combination of T-VEC (injected in the palpable tumor site) with intravenous pembrolizumab in 20 patients with advanced or metastatic sarcoma.[79] Patients had 13 different sarcoma histotypes and 60% of them had received three lines or more of therapy before enrollment. The combination was well tolerated with 20% of them experiencing treatment-related adverse events and demonstrated interesting efficacy with 35% ORR and a duration of response of 56.1 weeks. Interestingly, two responders to the combination had disease progression while receiving immunotherapy just before study enrollment, suggesting synergism between treatments. The small sample size of ancillary studies limited the ability to draw definitive conclusions.

Vaccine efficacy is based on the stimulation of the endogenous immune system of patients, for example, through the presentation of an antigen by dendritic cells stimulating CD8+ T cells. Although various vaccine therapies have been explored for sarcomas over the past 20years, overall the efficacy has been limited likely due to the other suppressive mechanisms in the immune microenvironment. Future studies of vaccines in combination with other therapies including ICIs may help to overcome these resistance mechanisms. LV305 is an NY-ESO-1 expression third-generation lentiviral vector designed to deliver RNA tumor antigens to dendritic cells, selectively targeting DC-SIGN (CD209) on the surface of immature human dendritic cells. To increase the efficacy of LV305, it was then combined with G305, including a full-length NY-ESO1 protein and a toll-like receptor 4 (TLR4) agonist as an adjuvant.[80] This strategy of alternative targeting of the same antigen was called “CMB305 regimen” and assessed in a phase Ib trial.[81] In this study, 64 patients had sarcoma among which 69.8% had greater than 75% NY-ESO-1 expression. The treatment was well tolerated and led to a 61.9% control rate in sarcoma with 26.2-month overall survival. A randomized phase II study was conducted in 89 patients with advanced/metastatic SS or myxoid liposarcoma, known to frequently express NY-ESO-1, to assess the combination of CMB305 and atezolizumab versus atezolizumab alone.[82] The combination

failed to significantly increase PFS and OS in the whole cohort even though there was evidence of benefit to a subset of patients who developed anti-NY-ESO-1 T-cell immune response. Given the overall lack of neoantigens as a resistance mechanism in sarcomas, future investigations of vaccines are warranted.

Macrophages and other myeloid cells are highly interesting targets for ongoing and future explorations. Dedicated studies with checkpoint inhibitors and CSF1R blockade aiming to repolarize M2 suppressive macrophages to M1 activated phenotypes are ongoing (NCT04242238), and there is a significant rationale for future investigation of targeting the CD47/SIRPα axis. A recent study highlighted variable expression and presence of macrophages across over 1200 specimens representing 24 sarcoma subtypes, with CD163+ M2 suppressive macrophages as the dominant phenotype, and preferentially present in nontranslocation sarcomas over other sarcomas.[83] CD47 staining was bimodal, with either absent or very high expression, which correlated with SIRPα expression. Subtypes with the highest expression of CD47/SIRPα on macrophages included angiosarcomas, chordoma, and pleomorphic liposarcomas. Interestingly, more than 50% of Ewing's sarcomas assessed had tumor positivity of SIRPα raising the question of an alternate function. Another study recently supported the importance of myeloid signatures in sarcoma,[84] again making this an area that should be prioritized in future clinical trials.

TRAIL-TNF axis—TRAIL is a cytokine member of the TNF superfamily, and TRAIL-R1 (death receptor 4) and TRAIL R2 (DR5) family have been shown to be expressed in a variety of sarcomas. Ongoing studies showed early promising outcomes with stimulatory agonists for DR5,[85] with plans for a dedicated phase 2 study in chondrosarcomas (NCT04950075). As of yet no combinations with checkpoint blockade have been planned but would have interesting rationale.

SUMMARY

In the past 5 years, we have seen tremendous growth in laboratory, translational, and clinical investigation that revitalizes the hope raised from Sir William Coley's initial observations in the 1890s that sarcomas could be susceptible to immune recognition and attack.[86] With a subset of sarcoma patients showing remarkable and durable responses to immune therapies even after the failure of numerous traditional treatments, it is tempting to imagine a future where the individual tumor and host genetic and immune factors can be assessed and treatments customized to overcome immune evasion. To reach this goal, we must continue to learn from every sarcoma patient treated on immune therapy clinical trials and increase collaboration in the laboratory and clinical research realms. By taking advantage of emerging genetic and immune datasets, and improving collaborative trial designs with novel agents and strategies, immunotherapy may well become a standard aspect of the sarcoma therapeutic armamentarium over the next years.

DISCLOSURE

All authors have no conflict of interest to declare.

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