Review

Role of Immunotherapy in Sarcomas

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Role of Immunotherapy in Sarcomas

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Abstract: Sarcomas are a group of malignancies of mesenchymal origin with plethora of subtypes. Given the sheer heterogeneity of various subtypes and rarity of the disease, management of sarcomas has been challenging with poor patient outcomes. Surgery, radiation therapy and chemotherapy have remained the backbone of treatment in patients with sarcoma. The introduction of immunotherapy has revolutionized the treatment of various solid and hematological malignancies. In this review, we discuss basics of immunotherapy and immune microenvironment in sarcomas, various modalities of immunotherapy such as immune checkpoint blockade, oncolytic viruses, cancer targeted antibodies, vaccine therapy and adoptive cell therapies like CAR T-cell therapy, T cell therapy, TCR therapy.

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1. Introduction

Sarcomas are a group of heterogeneous malignancies of mesenchymal origin with more than 100 histologic subtypes. They have diverse molecular, genetic, and clinical features and comprise 1% of adult malignancies [1–3]. They can be generally classified into two major types: soft tissue sarcomas (STS), which include more than 50 subtypes (with the most common being liposarcoma, leiomyosarcoma and undifferentiated pleomorphic sarcoma), and bone sarcomas (BS) (osteosarcoma, chondrosarcoma, and Ewing's sarcoma) [4,5] (Refer to Tables 1 and 2 for WHO classifications). Some of the sarcoma subtypes are extremely rare with limited current knowledge of their pathophysiology and treatment responses, and they are underrepresented in clinical trials.

Table 1. WHO Classification of Soft Tissue Sarcomas [61].

Adipocytic tumors	**Atypical lipomatous tumor** **Well-differentiated liposarcoma** **Liposarcoma, NOS** **Dedifferentiated liposarcoma** **Myxoid/round cell liposarcoma** **Pleomorphic liposarcoma**
Fibroblastic/myofibroblastic tumors	Dermatofibrosarcoma protuberans Fibrosarcomatous dermatofibrosarcoma protuberans Pigmented dermatofibrosarcoma protuberans Solitary fibrous tumor, malignant Inflammatory myofibroblastic tumor Low-grade myofibroblastic tumor Fibrosarcoma Myxofibrosarcoma Low-grade fibromyxoid sarcoma Sclerosing epithelioid fibrosarcoma
So-called Fibrohistiocytic tumors	Giant cell tumor of soft parts NOS Malignant tenosynovial giant cell tumour
Smooth muscle tumors	Leiomyosarcoma
Pericytic (perivascular) tumors	Malignant glomus tumor
Skeletal muscle tumors	Embryonal rhabdomyosarcoma Alveolar rhabdomyosarcoma

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	Pleomorphic rhabdomyosarcoma
	Spindle cell/sclerosing rhabdomyosarcoma
Vascular tumors	Retiform hemangioendothelioma
	Papillary intralymphatic angioendothelioma
	Composite hemangioendothelioma
	Pseudomyogenic hemangioendothelioma
	Kaposi sarcoma
	Epithelioid hemangioendothelioma
	Angiosarcoma of soft tissue
Chondro-osseous tumors	Soft tissue chondroma
	Extraskeletal osteosarcoma
Gastrointestinal stromal tumors	Gastrointestinal stromal tumor, malignant
Nerve sheath tumors	Malignant peripheral nerve sheath tumors
	Melanotic malignant nerve sheath tumor
	Granular cell tumor, malignant
	Perineurioma, malignant
Tumors of uncertain differentiation	Ossifying fibromyxoid tumor, malignant
	Stromal sarcoma, NOS
	Myoepithelial carcinoma
	Phosphaturic mesenchymal tumor, malignant
	Synovial sarcoma NOS
	Synovial sarcoma, spindle cell
	Synovial sarcoma, biphasic
	Epithelioid sarcoma
	Alveolar soft part sarcoma
	Clear cell sarcoma
	Extraskeletal myxoid chondosarcoma
	Extraskeletal Ewing sarcoma
	Desmoplastic small round cell tumor
	Perivascular epithelioid tumour, malignant (PEComa)
	Intimal sarcoma
Undifferentiated/unclassified sarcoma	Undifferentiated spindle cell sarcoma
	Undifferentiated pleomorphic sarcoma
	Undifferentiated round cell sarcoma
	Undifferentiated epithelioid sarcoma
	Undifferentiated sarcoma, NOS

Table 2. WHO Classification of Bone Sarcoma [61].

Chondrogenic tumors	**Chondrosarcoma**
	Dedifferentiated chondrosarcoma
	Mesenchymal chondrosarcoma
	Clear cell chondrosarcoma
Osteogenic tumors	Osteoblastoma
	Osteosarcoma
	Conventional osteosarcoma: chondroblastic, fibroblastic, osteoblastic
	Periosteal osteosarcoma
Osteoclastic giant cell rich tumors	Giant cell tumor of bone, malignant
Fibrohistiocytic tumors	Undifferentiated high grade pleomorphic sarcoma of bone (previously Malignant fibrous histiocytoma)
Notochordal tumors	Chordoma
	Dedifferentiated chordoma
Vascular tumors	Epithelioid hemangioendothelioma
	Angiosarcoma
Undifferentiated small round cell sarcoma of bone and soft tissue	Ewing sarcoma
	Round cell sarcoma with EWSR1 non-ETS fusion
	Sarcoma with BCOR genetic alterations
Fibrogenic tumors	Fibrosarcoma

Recently, incorporation of immunotherapies to treatment regimens has been heavily investigated and has revolutionized the treatment of solid tumors. Immune check point inhibitors (ICIs) have limited efficacy in sarcomas compared to other solid tumors, however it has shown some activity in certain subtypes. Moreover, clinical trials are heterogenous, as most have been basket trials

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with a variety of different sarcoma subtypes despite their unique biological characteristics, thus making it difficult to utilize ICIs in rare subtypes. There are several challenges with immunotherapy use in sarcoma due to tumor heterogeneity, the paucity of targetable antigens in sarcoma subtypes by therapeutic antibodies, vaccines, chimeric antigen receptors, and the lack of individualized trials for rare subtypes. However, combination of ICIs with other agents appears to have synergistic effects, and potential treatment options such as adoptive cell therapy and oncolytic viruses are emerging. In this review, we discuss the biological basis, current clinical trials, and future challenges of immunotherapy in advanced sarcomas.

2. Current Sarcoma Treatment Landscape

Treatment of STS can be based on the given subtype, such as STS of extremity, superficial/trunk or head and neck; retroperitoneal or intra-abdominal STS, desmoid tumors and rhabdomyosarcomas [6]. Patients should be evaluated and managed by a multidisciplinary team, including experienced pathologists, radiologists, medical oncologists, surgical oncologists, and radiation oncologists for consideration of systemic therapy, surgery, and/or radiation [6]. Conventional chemotherapy, including anthracycline-based regimens, is the standard treatment for most advanced and metastatic STS and non-anthracycline based regimens are preferred in angiosarcomas (AS) and perivascular epithelioid cell neoplasms [7–9]. A number of tyrosine kinase inhibitors (TKIs) have shown promising results in patients with certain histologic subtypes of advanced and metastatic STS. Pazopanib, a multitargeted tyrosine kinase inhibitor (multi-TKI), can be used as a single agent in metastatic nonlipogenic STS patients previously treated with anthracycline-based chemotherapy, or as a front line in advanced/metastatic STS patients who are not candidates for anthracycline-based regimens [10,11]. Other TKIs used in advanced STS include regorafinib (nonadiopocytic sarcoma and AS), sorafenib (desmoid tumors) and imatinib (dermatofibrosarcoma protuberans and gastrointestinal stromal tumors (GIST)) [12–15].

Treatment of BS can be vastly different depending on subtypes. Osteosarcomas (OS) are usually radiation resistant, and treatment involves wide excision with perioperative chemotherapy including doxorubicin, cisplatin, and high dose methotrexate [16]. On the other hand, Ewing's sarcomas (ES) are sensitive to radiation, and treatment usually involves perioperative chemotherapy with vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide with surgery with or without radiation [17]. Chondrosarcomas (CS) are chemotherapy and radiation resistant, and primary treatment is surgical resection [18]. Recently, TKIs like regorafenib, cabozantinib and apatinib have also been shown to be effective in OS [19]. The investigation of TKIs for other BS has not been well developed but it has shown some promising results in preclinical and early trials in ES and chondrosarcomas [19]. Currently, atezolizumab is the only immunotherapy drug approved by the Food and Drug Administration (FDA) for sarcomas and it is approved for unresectable or metastatic alveolar soft part sarcoma (ASPS) on December 9th, 2022 [20]. Pembrolizumab can be considered as a second line treatment for patients with certain subtypes of advanced or metastatic STS, including myxofibrosarcoma (MFS), undifferentiated pleomorphic sarcoma (UPS), cutaneous AS and undifferentiated sarcomas [6].

3. Cancer immunotherapy

Clinical benefits of immune enhancement in cancers have been well proven since the 1800s. Immunotherapy is the fifth pillar of cancer treatment after surgery, chemotherapy, radiation therapy and targeted therapy. In some cases, it has become the first line of treatment [21]. Cancer immunotherapies can be categorized based on the mechanism of action: (i) checkpoint blockade which removes the natural inhibitory signals of the immune system; CTLA-4 inhibitor (e.g., ipilimumab), PD-1 inhibitor (e.g., pembrolizumab, nivolumab), PD-L1 inhibitor (e.g., atezolizumab, avelumab, durvalumab) (ii) adoptive cell therapies including infusion of modified immune effector cells (T cells, Chimeric Antigen Receptor T cells (CAR T-cells), NK cells or TCR based therapy; (iii) cancer vaccines; (iv) oncolytic viruses; and (v) cancer targeted antibodies. The success of

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immunotherapy is significantly affected by the immunogenicity of tumor, tumor mutation burden and tumor microenvironment.

4. Immune Microenvironment and Biomarkers in Sarcoma

The tumor microenvironment (TME) is a primary location for cell-to-cell interactions around the tumor, signal transfer or delivery, and cytokine production. It plays an important role in tumor cells escaping the natural immune system and can similarly affect the efficacy of some immunotherapies. The components of the TME are tumor-associated macrophages (TAMs), pro-inflammatory cytokines, other immune checkpoint modulators, regulatory T-cells (Treg), immunosuppressive cytokines; for example, transforming growth factor-beta (TGF-beta), pro-angiogenic cytokine like fibroblast growth factor (FBGF), or vascular endothelial growth factor (VEGF). They are functioning through a complex pathway to maintain the tumor growth and to overcome the anti-cancer immune system.

Being a heterogeneous disease with multiple subtypes, sarcoma has a variety of genetic profiles and characteristics of tumor cells in each individual subtype [22]. The tumor mutational burden (TMB) and microsatellite instability (MSI) status have commonly been used to predict tumor response to immunotherapy. There is high variability of TMB among different subtypes of sarcoma. For example, a few sarcoma subtypes such as soft tissue rhabdomyosarcoma, alveolar, liposarcoma and synovial sarcoma have low TMB, whereas soft tissue angiosarcoma has high TMB with a median mutational burden of 3.8 mutations/Mb and 13.4% of cases having more than 20 mutations/Mb [22].

Another potential predictive factor used to evaluate utility of immunotherapy in sarcoma treatment are tertiary lymphoid structures (TLS), which refer to the organized aggregates of lymphoid cells forming around the tumor cells. Usually observed via use of immunohistochemistry (IHC), the aggregation of-B cell follicles, dendritic cells, helper T cells (CD4+) and cytotoxic T cells (CD8+) represents the TLS phenotype, which is frequently found in high immune sarcoma types and can predict better outcomes with immune checkpoint inhibitors (ICI) [23,24]. This hypothesis is supported in the PEMBROSARC trial, a multicohort phase II trial, which showed that the presence of TLS features in the TME was associated with a higher pembrolizumab treatment response [25]. Interestingly, a high infiltration of regulatory T (Treg) cells, which modulate the immune function, was found in TLS positive non-responder groups, and decreased the effect of pembrolizumab [25]**.** As such, the clinical impact of TLS is still controversial with limited known predictive value currently.

By analyzing bulk RNA transcriptome data from tumor infiltrating lymphocytes (TILs) in 85 osteosarcoma patients, researchers identified five different TIL marker genes. These genes were used to create a risk model with both prognostic and predictive value. In this model, varying levels of expression of these five genes were used to classify patients based on higher survival (“low risk”) or lower survival (“high risk”). Additionally, it was found that high risk tumors had a lower abundance of immune cell infiltration, whereas low risk tumors had a higher expression of immune checkpoint genes such as CTLA4 and LAG3, which could provide a positive predictive value in the response to immunotherapy for low risk patients [26]. The combination of IHC for TLS phenotype and the TIL molecular RNA signature could potentially be used together to provide enhanced prognostic and predictive models.

PD-L1 expression varies in different types of tumors. High PD-L1 expression is found in high grade dedifferentiated leiomyosarcoma [27]. However, there is no sufficient data to support PD-1 or PD-L1 as a predictive biomarker for ICI treatment. The efficacy of pembrolizumab was not related to the level of PD-L1 expression in the SARC028 trial [28]. In endometrial sarcoma, PD-L2 expression was associated with mismatch repair (MMR) proficient tumors and lower OS rates when compared to PD-L1 expression [29]. Similar findings were reported in uterine adenosarcoma, which also showed that PD-L1 expression did not correlate with density of TILs, but PD-L2 expression is positively correlated with TP53 mutation, which is associated with worse clinical outcomes [30].

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6. Immune Checkpoint Blockade

ICIs target tumor cells' known inhibitory signals to T cells and have demonstrated response in solid tumors. Immune checkpoint receptors including CTLA-4, PD-1, PD-L1, LAG-3 are inhibitory molecules present on the surface of immune cells, cancer cells and other supporting cells in the tumor microenvironment (TME) [31]. Sarcomas usually have low tumor mutational burden (TMB) and immunosuppressive TME, low PD-L1 expression, and only a few percent of these tumors are mismatch repair deficient; they are not considered immune sensitive tumors [1]. Even though response to ICIs in sarcomas is not high in general, there is some benefit in specific histological subtypes [1]. In addition, the role of PD-L1 expression in STS is unclear, as responses are seen even in the absence of PD-L1 expression [32].

Earlier trials with single-agent immunotherapy (anti PD-1 nivolumab, anti-CTLA-4 ipilimumab) failed to demonstrate significant anti-tumor activity [33,34]. One of the first ICI trials with positive results was the prospective single arm phase II trial SARC028, evaluating the anti-PD1 antibody pembrolizumab as a second line in 80 patients with either STS or BS [32]. This study demonstrated an objective response rate (ORR) of 18% in patients with STS with median progression free survival (PFS) and overall survival (OS) of 18 and 49 weeks, respectively [32]. One patient with UPS had a complete response (CR), but the benefit was limited to the patients with UPS and dedifferentiated liposarcoma (DDLPS), with minimal benefit in synovial sarcoma (SS), leiomyosarcoma (LMS) or BS [32]. However, this trial excluded rare STS subtypes, thus the efficacy of anti-PD1 in rare STS subtypes was not evaluated. The response of UPS to pembrolizumab was further confirmed in an expansion cohort of SARC028 with two CR and seven partial responses (PR) in the UPS cohort, but the response was not confirmed in the liposarcoma (LPS) cohort [35].

A phase II French AcSé trial evaluating the efficacy of pembrolizumab in different cohorts of patients with rare cancers, including the rarest sarcoma subtypes, showed an ORR of 15.3% with disease control rate (DCR) of 52.5% [36]. It demonstrated the highest response rates in ASPS with 50% ORR and in SMARCA4-deficient malignant rhabdoid tumor (SMRT) with 27% ORR. Other response rates were 8.8% in chordoma, 12.5% in desmoplastic small round cell tumors (DSCRT) and 3.2% in other histotypes [36].

A pooled analysis of clinical trials investigating anti PD-1/PD-L1 immunotherapy in patients with advanced STS including UPS, LPS, LMS, ASPS, reported that among 384 patients, 39.8% received anti-PD1/PD-L1 immunotherapy and had ORR of 15.1% and median PFS of 58.5% [26]. ASPS and UPS were among the highest responders (48.4% and 15.7%, respectively) and LPS and LMS among the lowest (7.3% and 6.9%, respectively) [37]. In the first line setting, a retrospective study of nivolumab with or without ipilimumab was evaluated in PD-L1 positive STS, demonstrating an ORR of 13% in the combination group vs 7% in the nivolumab group [38].

Targeting different immune checkpoints including PD-1, CTLA-4 and LAG-3 simultaneously is a promising approach to improve the effectiveness of immunotherapy. Lussier et al. showed that CTLA-4 expression was upregulated in T cells infiltrating PD-L1 antibody-resistant tumors in mice with metastatic OS, suggesting a potential synergic effect of anti CTLA-4 and PD-L1 blockade [39]. Combination checkpoint inhibition with nivolumab and ipilimumab was evaluated in previously treated patients with advanced STS in the phase II Alliance A091401trial with an ORR of 16%, and a median PFS and OS of 4.1 and 14.3 months, respectively, and better responses are seen with combination therapy and the best responses in UPS (33%), LMS (14.2), and AS (33%) [40].

Dual blockade of PD-1 and LAG-3 has also shown synergistic antitumor activity in preclinical models. A phase II basket trial of anti-PD1 spartalizumab plus anti-LAG3 LAG525 with a cohort of 10 sarcoma patients reported 40% DCR at 24 weeks [41]. Expansion criterion was not met, but the sarcoma cohort was not found to be futile [41]. Currently, the combination of nivolumab plus anti-LAG3 relatimab vs nivolumab alone is being investigated in advanced STS in ongoing phase II trial NCT04095208 (CONGRATS trial).

Tumor biomarker analysis of ASPS affirms the presence of PD-1/PD-L1 immune check point components, suggesting that immune check point inhibition could be beneficial in advanced ASPS [42]. A phase II trial of 43 evaluable patients with ASPS using anti-PD-L1 atezolizumab showed an

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ORR of 37.2% with median duration of response (DOR) of 16.5 months [42]. Recently updated results with 52 patients confirmed the ORR of 37% with DOR of 24.7 months, and PFS of 20.8 months [43]. The FDA approved atezolizumab for unresectable or metastatic ASPS on December 9th, 2022 [20].

Given the lack of randomized phase III trials and limited therapeutic alternatives for patients who progressed on chemotherapy, ICI could be considered especially in patients with UPS, DDLPS as well as ASPS. Dual immune blockades appear to show higher response rates and may be considered in selected patients. Further details and summary of ICI trials are listed in Table 3.

Table 3. Results of Selected Trials of Immunotherapy in Sarcoma.

Clinical Trial/Design	Phase	Agent/Intervention	Indication/Prior lines of treatment	Evaluated patients (n) and Tumor Subtypes	ORR (%)	PFS (weeks (w) or months (m))	OS (w or m)	Outcomes in subtypes/Notes
ICI monotherapy or combination								
Maki et al. 2013 [33]	Phase II	ipilimumab	Locally recurrent or metastatic SS, at least 1 prior line treatment	6 SS	0%	1.85 m	8.75 m	
Tawbi et al. (SARC028) 2017 [32]	Phase II	pembrolizumab	Advanced/metastatic STS or bone sarcoma, at least 1 prior line treatment	40 BS cohort (22 OS, 13 ES, 5 CS)	-	8 w	52 w	1 PR in CS and 1 PR in OST
				40 STS cohort (10 LMS, 10 LPS, 10 SS, 10 UPS)	-	18 w	49 w	1 CR and 3 PR In UPS< 2 PR In LPS, 1 PR in SS
Ben-Ami et al. 2017 [34]	Phase II	nivolumab	Advanced or metastatic uterine LMS, at least 1 prior line of treatment	12 LMS	0%	1.8 m	-	
D' Angelo et al. Alliance A091401 2018 [40]	Phase II	nivolumab/ipilimumab vs nivolumab	Advanced or metastatic BS and STS, at least 1 prior line of treatment	Nivolumab/ipilimumab– 42 (3 AS, 4 BS, 14 LMS, 2 LPS, 6 SCS, 2 SS, 6 UPS/MFH, 1 unspecified sarcoma, 4 others)	16%	4.1 m	10.7 m	Response in uterine LMS, non-uterine LMS, MFS, UPS/MFH, AS
				Nivolumab– 43 (5 BS, 15 LMS, 3 LPS, 2 unspecified sarcoma, 5 SCS, 2 SS, 5 UPS, 6 others	5%	1.7 m	10.7 m	1 PR in ASPS and 1 PR in non-uterine LMS
Uboha et al. 2019 [41]	Phase II	spartalizumab + LAG525 (anti-LAG3)	Advanced solid tumors and hematologic malignancies	10	CBR 40%	-	-	Sarcoma cohort did not meet the expansion criterion
Zhou et al. 2020 [62]	Retrospective	nivolumab + ipilimumab	Advanced or metastatic STS, 87% received at least 1 prior line	38 (9 LMS, 8 Sarcoma NOS, 6 LPS, 5 MFS, 3 MPNST, 2 SFT, 1 Breast AS, 1 FDFP, 1 RMS, 1 SS)	15%	2.7m	12 m	CR in 1 MFS 1 PR in each MPNST, SFT, MFS, DDLS, and sarcoma NOS

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Naqash et al. 2021 [42]	Phase II	pembrolizumab	Advanced or metastatic ASPS	43	37.2%	-	-	
Blay et al. French AcSé 2021 [36]	Phase II	pembrolizumab	Advanced rare sarcoma	98 (34 chordoma, 14 ASPS, 11 SMRT, 8 DSCRT, 31 other histotypes)	15.3	2.75 m	19.7 m	Highest ORR In chordoma, ASPS, SMRT, DSCRT
Delyon et al. 2022 [63]	Phase II	pembrolizumab	Classic/endemic Kaposi sarcoma with extensive cutaneous extension, 71% had at least 1 prior line	17 (8 classic KS and 9 endemic KS)	71%	-	-	
Zer et al. 2022 [64]	Phase II	ipilimumab and nivolumab	Classic Kaposi sarcoma, at least 1 prior line of treatment	11	45%	not reached	-	
Somaiah et al. 2022 [65]	Phase II	durvalumab + tremelimumab.	Advanced or metastatic sarcoma (BS and STS), 91% had at least 1 prior line	57 (3 DDLPS, 2WDLPS, 1 PLS, 5 AS, 5 LMS, 5 UPS, 5 SS, 1 CDOS, 4 COS, 10 ASPS, 5 chordomas, 11 other sarcomas)	12%	2.8 m	21.6 m	ASPS ORR 40%
ICI combination with TKI								
Schoffski et al. 2016 [66]	Ia/Ib	pembrolizumab + olaratumab (monoclonal antibody against platelet derived growth factor receptor alpha)	Advanced or metastatic STS, 92% had at least 1 prior line	28	21.4%	2.7 m	14.8 m	
Paoluzzie et al. 2016 [67]	Retrospective study	durvalumab + pazopanib	Metastatic STS and BS, median 2 prior lines of treatment	28 (24 STS, 4 BS with 24 evaluable patients)	10%	-	-	3 PR (1 DDCS with nivolimab alone), 1 EpS, 1 MOS)
Wilky et al. 2019 [48]	Phase II	pembrolizumab + axitinib	Advanced or metastatic STS, 81% with at least 1 prior line of treatment	33 (12 ASPS, 6 LMS (4 uterine), 5 High grade PS, 2 DDLPS, 8 other histotypes)	25%	4.7 m	18.7 m	ASPS ORR 50%,
Xie et al. APFAO trial 2020 [49]	Phase II	camrelizumab + apatinib	Advanced or metastatic OS, at least 1 prior line of treatment	43 (OS including osteoblastic, chondroblastic, fibroblastic and small cell)	20.1%	6.2 m	11.3 m	
Palmerini et al. IMMUNOSARC 2020 [46]	Phase II	nivolumab + sunitinib	Advanced BS cohort, at least 1 prior line of treatment	40 (17 OS, 14 CS, 8 ES, 1 bone UPS, 4 DDCS)	5%	3.7 m	14.2 m	1 CR in DDCS and 1 PR in OS

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Martin-Broto et al. IMMUNOSARC 2020 [68]	Phase I/II	nivolumab + sunitinib	Metastatic STS, at least 1 prior line of treatment	52 (9 SS, 8 UPS, 7 clear cell sarcoma, 7 SFT, 7 EpS, 5 AS, 4 ESMCS, 4 ASPS, 1 EHET)	21%	5.6 m	-	1 CR in AS, 2 PR in ASPS, 1 PR in ESMCS and 1PR in SS
Kim et al. 2021 [50]	Phase II	durvalumab + pazopanib	Advanced or metastatic STS, at least 1 prior line of treatment	46	28.3%	7.7 m	-	Objective responses in ASPS, AS, UPS, DSRCT
Cousin et al. REGOMUNE 2022 [69]	Phase II	avelumab + regorafenib	Advanced or metastatic STS, at least 1 prior line of treatment	43 (22 LMS, 9 SS, 4 LPS, 4 UPS, 10 other subtypes)	9.3%	1.8 m	15.1 m	
Allred et al. Alliance A091902 trial 2023 [70]	Phase II	nivolumab with carbozantinib	Advanced AS, previously treated	18 (AS including 12 cutaneous, 1 liver, 2 breast, 6 others)	72%	9.6 m	20.5 m	
Eulo et al. 2023 [71]	Phase II	nivolumab/ipilimumab + cabozantinib N/I + C	Metastatic STS that lacks translocation, at least 1 prior line of treatment	69 (N/I + C arm)	11%	5.4 m	-	
				36 (C only arm)	6%	3.8 m	-	
ICI combination with chemotherapy								
Toulmonde et al. 2018 [53]	Phase II	pembrolizumab + metronomic cyclophosphamide	Advanced or metastatic STS, 97% with at least 1 prior line of treatment	50 (15 LMS, 16 UPS, 16 other sarcomas, 10 GIST)	2%	1.4 m	-	
Italiano et al. Amended PEMBROSARC 2022 [25]	Phase II	pembrolizumab with metronomic cyclophosphamide	TLS-positive advanced STS, 63% had at least 1 prior line of treatment	35 (12 WDLPS/DDLPS, 4 LMS, 6 UPS, 3 EpS, 10 other histotypes)	30%	6m PFS 40%	-	PR: 5 in DDLPS, 3 EpS, 1 LMS SD: 6 DDLPS, 1 FMS, 1 MFS, 1 uterine LMS, 1 UPS
Nathenson et al. 2020 [72]	Phase II	pembrolizumab + eribulin	Metastatic STS, at least 1 prior line of treatment	19 LMS (11 uterine LMS)	5.3%	11.1 w	-	
Smrke et al. 2021 [73]	Phase I	pembrolizumab + gemcitabine	Advanced or metastatic LMS, UPS	13 (2 UPS, 11 LMS)	-	5.1 m	-	LMS - DCR 73% (8 SD, 3 PD) UPS - DCR 100% (2 PR)
Wagner et al. 2022 [74]	Phase I/II	avelumab + trabectedin	Advanced or metastatic LPS and LMS, 86% had at least 1 prior line of treatment	35, only 23 evaluable (24 with LMS, 11 with LPS	13%	8.3 m	27 m	LMS 4 PR, 9 SD LPS 7 SD
Toulmonde et al. 2022 [75]	Phase Ib	durvalumab + trabectedin	Advanced or metastatic STS cohort, at least 1 prior line of treatment	16 (6 LMS, 2 DDLPS, 8 others)	7%	12m PFS 14.3%	-	
Adnan et al. Gallant trial 2022 [76]	Phase II	nivolumab + metronomic gemcitabine,	Advanced or metastatic STS, at least 1 prior	39 (15 LMS, 4 PS, 4 SS, 3 LPS, 3 OS, 10 others)	20.5%	4.6 m	6.2 m	mPFS 2 m historically in previously

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		doxorubicin, and docetaxel	line of treatment					treated patients
Andreou et al. NITRA-SARC 2023 [57]	Phase II	nivolumab + trabectedin	Advanced or metastatic STS, at least 1 prior line of treatment	Group A – 43 (28 LMS and 15 LPS)	-	5.5 m	18.7 m	
				Group B – 49 (12 UPS, 11 SCS, 6 FMS, 5 SS, 4 EpS)	-	2.3 m	5.6 m	
Beveridge et al. ImmunoSarc 2 Cohort 7b 2023 [77]	Phase Ib	doxorubicin and dacarbazine plus nivolumab and nivolumab maintenance 1 year	Advanced or metastatic LMS, anthracycline naïve patients	16 LMS	56%	8.67 m	-	
ICI as front line								
Pollack et al. 2020 [54]	Phase I/II	pembrolizumab + doxorubicin	Anthracycline naïve sarcoma Excluding ES, ARMS, ERMS, 76% with no prior line of treatment	37 (11 LMS, 4 DDLPS, 3 CCCS, 3 UPS, 2 SFT, 2 ESS, 2 EHET, 8 other histotypes	19%	8.1 m	27.6 m	
Livingston et al. 2021 [55]	Phase II	pembrolizumab + doxorubicin	Anthracycline Naïve advanced STS, 86.7% had no prior treatment	28 (7 LPS, 10 LMS, 1 SS, 4 UPS, 2 AS, 6 other histotypes)	36.7%	5.7 m	17 m	The most clinical benefit in UPS, EpAS, LMS, LPS
Maleddu et al. 2023 [58]	Phase II	doxorubicin + anti-CTLA-4 zalifrelimab and anti-PD1 balstilimab	Advanced or metastatic STS, no prior doxorubicin or ICI	28	36%	25.6 m	-	Responses seen in IS, AS, MPNST, LPS, LMS, ESS, UPS, and SEpF.
Gordon et al. SAINT Trial 2023 [78]	Phase I/II	ipilimumab + nivolumab and trabectedin	Advanced or metastatic STS, treatment naive	101 (14 LPS, 26 LMS, 9 UPS, 7 RMS, 5 SS, 4 clear CS, 4 PS, 4 MFS, 3 PNST, 3 MLS, 2 carcinosarcoma, 2 DSRCT, 2 sarcoma NOS)	25.3%	6.7 m	24.6 m	
Chen et al. 2021 [38]	Retrospective	nivolumab + ipilimumab vs nivolumab	Metastatic STS (100% PD-L1 positive tumors – PD-L1 expression >1%), treatment naive	74 - Nivolumab and ipilimumab arm -(43 non-uterine LMS, 20 LPS, 11 SS)	-	4.1 m	12.2 m	
				76 - Nivolumab arm (40 non-uterine LMS, 22 LPS, 14 SS)	-	2.2 m	9.2 m	

Alveolar rhabdomyosarcoma – ARMS; Alveolar soft part sarcoma – ASPS; Angiosarcoma – AS; Desmoplastic small round cell tumor - DSRCT; Dedifferentiated chondrosarcoma – DDCS; Epithelioid sarcoma – EpS; Extraskeletal myxoid chondrosarcoma – ESMCS; Breast angiosarcoma – breast AS; Chondrosarcoma – CS; Chondroblastic osteosarcoma – CDOS; Clear cell chondrosarcoma – CCCS; Clear cell sarcoma – Clear CS; Conventional osteosarcoma – COS; Dedifferentiated liposarcoma – DDLPS; Embryonal rhabdomyosarcoma – ERMS; Epithelioid Angiosarcoma – EpAS;

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Endothelial stromal sarcoma – ESS; Epithelioid hemangioendothelioma – EHET; Epithelioid sarcoma – EpS; Fibromyxoid sarcoma – FMS; Fibrosarcomatous dermatofibrosarcoma protuberans – FDFP; Gastrointestinal stromal tumor – GIST; High grade pleomorphic sarcoma – High grade PS; Intimal sarcoma – IS; Pleomorphic liposarcoma – PLS; Pleomorphic sarcoma – PS; Malignant fibrous histiocytoma – MFH; Malignant peripheral nerve sheath tumor – MPNST; Maxillary osteosarcoma – MOS; Myxofibrosarcoma – MFS; Leiomyosarcoma – LMS; Liposarcoma – LPS; Osteosarcoma – OS; Rhabdomyosarcoma – RMS; Sclerosing epithelioid fibrosarcoma – SEpF; Spindle cell sarcoma – SCS; Smarca4 deficient malignant rhabdoid tumor – SMRT; Solitary fibrous tumor – SFT; Synovial sarcoma – SS; Undifferentiated pleomorphic sarcoma – UPS; Well-differentiated liposarcoma – WDLPS. ORR – objective response rate; OS – overall survival; PFS –progression free survival.

6. Combination of ICI with Tyrosine Kinase Inhibitors

The quest to find the optimal response of ICI in sarcomas has led to the combination of ICI with targeted therapies, mainly anti-angiogenic and multi TKIs. Preclinically, the normalization of abnormal tumor vessels and increased infiltration of immune effector cells into tumors by anti-angiogenic TKIs has shown to enhance the efficacy of ICI [44]. In addition to blocking the immune-suppressive effect of vascular epidermal growth factors (VEGF), multi TKIs seem to make favorable immune modulating effect by decreasing the arrival of myeloid derived suppressor cells and tumor associated macrophages, as well as increasing the infiltration of dendritic cells, natural killer cells and CD8+ lymphocytes, potentially making multi-TKIs a reasonable combination with ICIs [45].

The combination of nivolumab and sunitinib in advanced BS was evaluated in the phase I/II trial IMMUNOSARC, with ORR of 5% with 1 CR in dedifferentiated chondrosarcoma (DDCS), 1 PR and 22 SD and PFS of 3.7 months [46]. In this same trial, an advanced STS cohort was also evaluated and found to have an ORR of 21% with 1 CR in AS, 5 PR, and 33 SD among 46 patients and a median PFS of 5.6 months [47]. On the other hand, pembrolizumab in combination with axitinib demonstrated promising responses in a phase II trial of 33 advanced sarcoma patients among which 51% were previously treated [48]. It showed an ORR of 25% (8 PR) and PFS of 4.7 months, with the most benefit seen in ASPS who had 50% ORR with PFS of 12.4 months [48]. The benefit was thought to be due to high tumor infiltrating lymphocytes and PD-L1 expression in ASPS tumors [48]. Another anti-PD1 camrelizumab was evaluated with apatinib in the phase II APFAO trial of 43 patients with chemotherapy-refractory OS and showed an ORR of 20.9% and PFS of 6.2 months with the most PFS observed in patients with lung metastasis and with PD-L1 tumor proportion score of 5% [49].

The combination of durvalumab with pazopanib in previously treated patients with advanced STS showed an ORR of 28.3% and median PFS of 7.7 months with objective responses in ASPS, AS, UPS, and dedifferentiated chondrosarcoma (DSRCT) [50,51]. Upon further analysis, tumors with high CD20+B cell infiltration and vessel density were reported to have a longer PFS and a better response than those with low B cell infiltration and vessel density [51].

Combinations of ICI and TKIs could be considered in patients with advanced STS after progression on standard chemotherapy. On the other hand, the effectiveness of this combination in BS is not very promising. Further investigations are needed to compare the clinical benefits of these combinations with single ICI or TKI monotherapy, including the utility in BS. Also, they can be potentially considered to be used as a front-line treatment in selected patients unfit for anthracycline based chemotherapy. Further details and summary of trials for combination of ICI and TKIs is listed in Table 3.

7. Combination of ICI with Conventional Chemotherapy

The rationale for combination of chemotherapy with ICI is that the induction of cell death by chemotherapy could synergize with immunotherapy and make ICI more effective [52]. Cytotoxic drugs can result in DNA damage leading to cell death, release of immunostimulatory signals known as damage-associated molecular patterns (DAMPs) and proteins that work as "danger signals", with eventual upregulation of PD1 and enhancement of effector lymphocytes 'activity [19].

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In the phase II PEMBROSARC trial of pembrolizumab with metronomic cyclophosphamide in 50 patients with locally advanced or metastatic sarcomas (both treatment naive and pretreated patients), including LMS, UPS and GIST showed limited activity, with just one PR in a patient with solitary fibrous tumor (SFT) with PD-L1 expression greater than 10% in immune cells [53]. This trial demonstrated that the clinical benefit of ICI with chemotherapy is very limited in an unselected population. An amended study of PEMBROSARC, which included 35 patients with **TLS**-positive advanced STS showed a 6-month non-progression rate and ORR of 40% and 30%, respectively, compared with 4.9% and 2.4% respectively in the previous cohort of PEMBROSARC study [25]. Thus, TLS presence in the advanced STS could be a potential predictive biomarker to improve the selection of patients for the treatment of ICI with chemotherapy.

The first combination phase I/II trial of pembrolizumab with doxorubicin in 37 anthracycline-naive patients with advanced STS demonstrated an ORR of 19% (7 PR), PFS of 8.1 months and OS of 27.6 months, with more prominent results in UPS and DDLPS subtypes [54]. Even though it failed to meet its primary endpoint with response rate of 29%, combination therapy was associated with longer PFS than doxorubicin alone (8.1 months vs. 4.1 months) [54]. Another trial of pembrolizumab and doxorubicin in advanced STS showed that patients with PD-L1 ≥ 5% had a 3 times greater ORR (63.6%) than those with PD-L1 <5% [55]. PD-L1 expression was not found to be associated with improved PFS or OS but was associated with improved ORR [55].

LMS and LPS are usually resistant to PD-1/PD-L1 inhibition, likely due to the infiltration of high levels of immunosuppressive tumor-associated macrophages (TAMs) [56]. Trabectedin could influence TME and reduce TAMs, thus improving antitumor adaptive immunity to anti-PD1 therapy [57]. Trabectedin in combination with anti-PD-L1 avelumab in a phase I/II study of patients with advanced LPS and LMS showed 2 PR and 11 SD, with 6 month PFS of 50.1% [56]. In the phase II NiTraSarc trial, nivolumab with trabectedin are evaluated as a second line treatment in anthracycline pretreated advanced STS patients (Group A with advanced LPS or LMS and Group B with other sarcomas including pleomorphic, spindle cell, fibromyxoid, synovial and epithelial sarcoma) [57]. In the late combination cohort (LCC), patients are treated with 3 cycles of trabectedin followed by trabectedin plus nivolumab whereas early combination cohort (ECC) started combination treatment at cycle 2 [57]. After a median follow up of 16.6 months, PFS in group A was 47.6% (60% in LCC vs 36.4% in ECC), and 14.6% in group B. Median PFS was higher in group A compared to group B (5.5 vs 2.3 months) and longer in LCC vs ECC (9.8 vs 4.4 months) [57]. OS was much higher in group A vs group B (18.7 vs 5.6 months), and longer in LCC vs ECC (24.6 vs 13.9 months) [57]. It confirmed the activity of trabectedin followed by combination with nivolumab in LPS and LMS [57].

Results of a phase II trial of doxorubicin with anti-CTLA-4 zalifrelimab and anti-PD1 balstilimab as first and second line treatment in 28 patients without prior doxorubicin or ICI were recently reported [58]. The study had a two-stage design, with stage 1 giving a priming dose of zalifrelimab and balstilimab in cycle 1 prior to adding doxorubicin at cycle 2, and stage 2 giving all drugs at cycle 1. ORR was 36% and DCR was 86%, with DOR of 12.8 weeks in overall population [58]. Patients who received ICI priming at cycle 1 prior to chemotherapy had a better 6-month PFS (56.3 vs 25%), ORR (56% vs 8.3%) and DCR (94% vs 75%) compared to stage 2 [58].

The combination of ICI and chemotherapy seems to respond better in specific histologic subtypes such as LPS, LMS and UPS. However, it is difficult to obtain meaningful results given the heterogeneity in the selections of patients and difficulty confirming therapeutic benefit of immunotherapy to chemotherapy without randomized trials. Moreover, further investigations are needed to evaluate the sequence of priming with either ICI or chemotherapy to find the most effective treatments. Phase III trials comparing these regimens to the standard treatment would be needed to confirm these findings and the utilization of ICI and chemotherapy as a front-line systemic treatment in advanced sarcomas, especially STS. Moreover, this combination is not very well investigated in BS. Further details and summary of trials for combination of ICI and chemotherapy is listed in Table 3.

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8. Combination of Immunotherapy with Local Radioation Therapy

Radiation may produce neoantigens that enhance the immunogenicity of tumors with low TMB, making them sensitive to ICI in a T-cell dependent manner [1]. A randomized phase II non-comparative trial evaluating neoadjuvant radiation with nivolumab alone or nivolumab with ipilimumab in 24 surgically resectable patients with DDLPS or UPS, showed significant clinical activity in UPS with a median pathological response of 95%, while it was 22.5% in the DDLPS cohort; with responses being similar irrespective of the addition of ipilimumab [59]. It was found that radiation therapy in UPS increased tumor infiltrating immune cells and tumor PD-L1 expression [59,60]. Combination of ICI with radiation therapy is currently being investigated in several trials (see Table 4).

Table 4. Currently Ongoing Selected Clinical Trials for Immunotherapy in Sarcomas.

Phase	NCT number/trial name	Status	Conditions	Interventions
ICI				
Phase I/II	NCT03138161 SAINT	Recruiting	Unresectable or metastatic STS as first line treatment	trabectedin + ipilimumab + nivolumab
Phase II	NCT04095208	Recruiting	Advanced or Metastatic STS (TLS +)	nivolumab + relatimab vs nivolumab
Phase II	NCT04802876 ACROPOLI (SOLTI-1904)	Recruiting	Across multiple cancer types with PD1-high mRNA Expressing Tumors - Include Sarcoma Cohort	spartalizumab + tislelizumab
ICI with TKIs				
Phase II	NCT04784247	Recruiting	Advanced STS	lenvatinib + pembrolizumab
Phase II	NCT05182164	Recruiting	Advance sarcomas: ES, OS, UPS	pembrolizumab + carbozantinib
Phase II	NCT04551430	Active, not recruiting	Metastatic STS	cabozantinib + nivolumab + ipilimumab
ICI with Chemotherapy				
Phase II	NCT03899805	Active, not recruiting	STS (LPS, LMS, UPS)	eribulin + pembrolizumab
Phase II	NCT04535713 GALLANT	Recruiting	Advanced sarcoma	metronomic gemcitabine + doxorubicin + docetaxel + nivolumab
Phase I/II	NCT05876715 LINNOVATE	Recruiting	Advanced STS	lurbinectedin + nivolumab + ipilimumab
Phase I/II	NCT04577014	Recruiting	Advanced STS	retifanlimab + gemcitabine + docetaxel
Phase II	NCT04028063	Recruiting	Advanced STS	doxorubicin + zalifrelimab - AGEN1884 + balstilimab – AGEN2034
ICI with Radiation Therapy				
Phase I	NCT05488366	Recruiting	Metastatic STS	pembrolizumab + Radiation Therapy
Phase II	NCT03307616	Active, no recruiting	Recurrent or resectable DDLPS and UPS before surgery	nivolumab +/- ipilimumab + Radiation Therapy
Phase I/II	NCT03116529	Active, not recruiting	High risk STS	durvalumab + tremelimumab + Radiation + Surgery

Retrieved from www.clinicaltrials.gov on October 6, 2023.

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Table 4. Ongoing clinical trials for CAR T-cell therapy in Sarcoma:.

Trial Number	Phase	Intervention	Disease
NCT01953900	Phase I	Anti-GD2 T-cells in combination with a varicella zoster vaccine and lymphodepleting chemotherapy	GD2 positive sarcoma and neuroblastoma in relapsed or refractory setting
NCT04995003	Phase I	Anti-HER2 CAR T-cells in combination with an immune checkpoint inhibitor drug (pembrolizumab or nivolumab)	HER 2 positive Sarcoma in patients disease progression or recurrence after at least one prior systemic therapy
NCT02107963	Phase I	Administering escalating doses of autologous anti-GD2-CAR T-cells	Osteosarcoma, GD2+ solid tumors that recurred or progressed on treatment
NCT00902044	Phase I	Anti-HER2 CAR T-cells with fludarabine and cyclophosphamide	Refractory HER2-positive sarcoma or metastatic HER2-positive sarcoma with disease progression after receiving at least one prior systemic therapy
NCT03721068	Phase I	Anti GD2 CAR T-cells, fludarabine and cyclophosphamide	Relapsed refractory osteosarcoma and neuroblastoma

Table 4. Ongoing Trial for Cancer Vaccine Therapy in Sarcoma.

NCT	Phase	Intervention	Disease
NCT01241162	Phase I	Mature DC pulsed with peptides derived from NY-ESO-1, MAGE-A1, and MAGE-A3 for vaccine production.	Relapsed refractory Ewings sarcoma, osteogenic sarcoma, rhabdomyosarcoma or synovial sarcoma

It is difficult to determine the most immune sensitive sarcoma subtypes given the heterogeneity of sarcomas, the limited numbers of patients enrolled, the inconsistencies in the designs and results of various trials, lack of phase III randomized clinical trials, lack of representatives of rare histology subtypes, and the lack of validated biomarkers for ICIs. Currently there is a need for trials with better designs and individualized studies investigating each group of sarcomas that share common biological characteristics.

9. Adoptive Cell Therapy

Adoptive cell therapy (ACT) is a new and innovative strategy that uses the immune system to target cancer cells. It has the potential to induce a durable response in tumors, and promising results have been seen in hematological malignancies and some solid tumors. ACT involves extraction of immune cells from a patient's blood, tumor tissue or healthy donor via leukapheresis. The cells are then genetically engineered ex-vivo to make them targeted towards specific tumor cells, and then expanded prior to reinfusion in the patient. T-cells have the ability to both kill tumor cells directly and activate additional immune cells, subsequently eliciting an endogenous immune response. Three classic examples of ACTs used for cancer immunotherapy are:

A. T-cell therapy
B. Chimeric antigen receptor T-cell therapy (CAR-T)
C. T-cell receptor-based therapy (TCR)

A. T-Cell Therapy

T-cell based therapy is comprised of tumor infiltrating lymphocytes (TILs), which are extracted from the tumor, activated ex-vivo, expanded and reinfused in the patient in combination with immune enhancing adjuvants, such as interleukin-2 (IL-2), to induce a durable immunological response against the tumor cells. Prior to reinfusion of these TILs, the patient receives a

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lymphodepleting chemotherapy regimen, such as cyclophosphamide and fludarabine, to deplete the innate T-cells that may suppress the proliferation of the infused T-cells in the body [79]. In contrast to engineered TCRs and CAR-T cells, this is the only ACT technique with multiple T-cell receptor clones able to target the antigenic heterogeneity of sarcoma[80]. The role of TILs in cancer immunotherapy has been studied in various cancers, such as renal cell carcinoma, breast cancer, colon cancer, melanoma amongst various others [81]. Earliest studies dating back to three decades ago by Balch et al. reported TILs were present in about 35% of patients with sarcoma particularly gastrointestinal stromal tumor (GIST), STS, Ewing's sarcoma (ES), osteosarcoma and uterine sarcomas however their potential consideration as predictive markers is unclear based on the current data [81]. This approach of infusing ex-vivo expanded TILs was found to have striking efficacy in melanoma, with durable response rates and long-term survival benefits [82].

Mullinax at al. conducted a study on 70 patients with soft tissue sarcoma (STS) and demonstrated the feasibility of creating TIL cultures using a rapid expansion protocol. The study showed that TILs demonstrated tumor-specific reactivity by IFNγ release assay in 51 samples. The tumor-specific activity was noted in 56.3% of patients (9/16) using the fragment method (tumor fragments were minced into pieces ~1 mm^3 in size) and in 40% (14/35) using the digest method (tumor tissue was processed into a single cell suspension using both mechanical and enzymatic disruption) ($P = 0.37$ comparing fragment vs. digest methods)[83]. In a retrospective study conducted by Zhou et al., 60 patients with chemotherapy-resistant metastatic osteosarcoma were enrolled, and a combined approach with adoptive TIL and anti-PD1 therapy was investigated. The results were encouraging with an ORR of 36.7%, a DCR of 80%, and a median PFS of 5.8 months. Overall, OS was 23.7 months in responders versus 8.7 months in non-responders ($p < 0.0001$) [84].

However, despite interesting and promising preclinical and retrospective data, further research is required to understand and navigate the challenges still faced by the TIL therapy, especially in sarcomas, given the substantial heterogeneity between different subtypes.

A. CAR T-cell Therapy

CAR (chimeric antigen receptor) T-cell therapy is a type of adoptive cell therapy that aims to modify the DNA of a patient's T-lymphocytes in order to enable them to selectively target and eliminate cancer cells. The identification of tumor specific antigens for CAR T-cell targeting is challenging in solid tumors, given intense antigenic heterogeneity due to their polyclonal expansion and accumulative mutations, which makes it hard to find homogeneously expressed targets, particularly without unacceptable toxicity.

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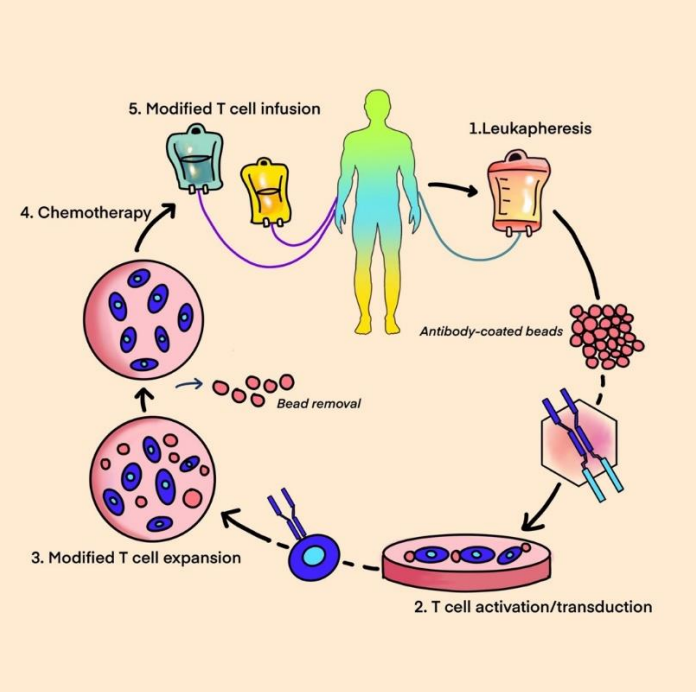

Figure 1. CAR T-cell- Leukapheresis, T cell modification, expansion, and CAR T-cell infusion.

Chimeric antigen receptor structure consists of: (Figure 2)

1. An antigen-recognition domain- a single-chain variable fragment (scFv) as a part of a genetically engineered monoclonal antibody that targets the selected tumor antigen.
2. A hinge that links a recognition site to the transmembrane domain bridging the membrane.
3. An intracellular domain that is critical for T-cell receptor signaling [85].

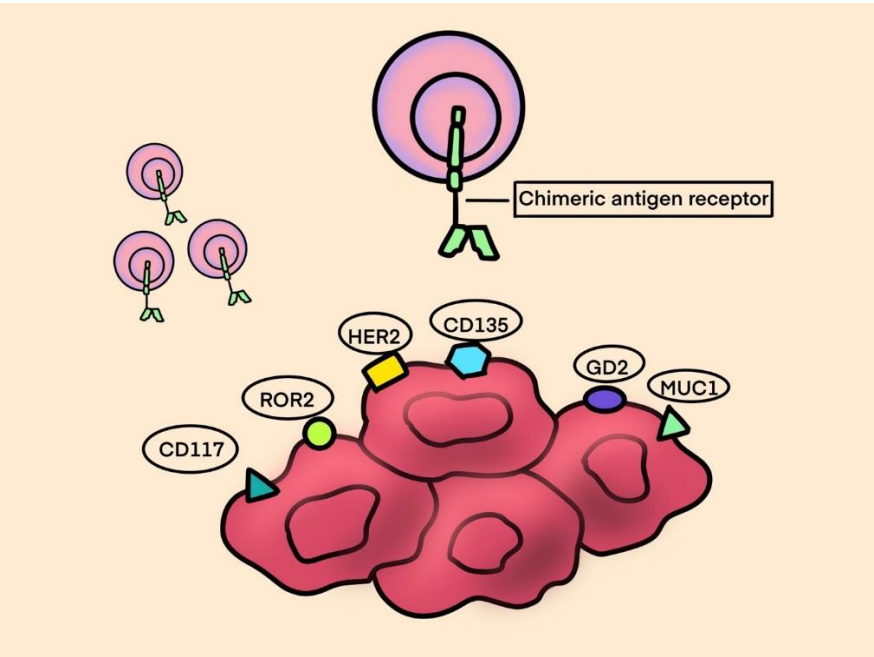

Figure 2. CAR T-cell structure.

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The positive results obtained in clinical trials using CAR T-cell therapy for treatment of B-cell lymphomas and acute lymphoblastic leukemias led to the extension of study of CAR T-cells in treatment of various types of sarcomas. GD2 (diasialoganglioside) has been considered an attractive target for cancer immunotherapy given it is over-expressed on various tumors, including neuroblastoma, melanoma, osteosarcoma, ES, and rhabdomyosarcoma, while it is poorly expressed in normal tissue. T-cells expressing the first generation anti-GD2 chimeric antigen receptors (CARs) were safe and mediated modest antitumor activity in some patients with refractory neuroblastoma[86]. Clinical trials testing the use of anti-GD2 CAR T-cells in patients with sarcomas and other GD-2 positive solid tumors are currently ongoing (See Table 1).

Another important phase I/II trial tested escalating doses of T-cells expressing a HER2-specific chimeric antigen receptor in patients with recurrent/refractory HER2 positive sarcoma[87]. This study demonstrated that the CAR T-cells could persist for 6 weeks without major toxicities, setting the stage for ongoing studies that combine anti-HER2 CAR T-cells with other immunomodulatory approaches to enhance their expansion and persistence [88]. Another important Phase I clinical trial in sarcoma is aimed at testing the combination of anti-HER2 CAR T-cell therapy in combination with immune checkpoint blocking agents such as pembrolizumab or nivolumab (NCT04995003). These patients are typically pretreated with lymphodepleting agents such as cyclophosphamide and fludarabine prior to infusion of CAR T-cells targeting the HER2 receptor. One week after the patient receives the HER2 CAR T-cells, they will begin pembrolizumab every three weeks or nivolumab every two weeks. This study is currently active and recruiting.

A. TCR therapy

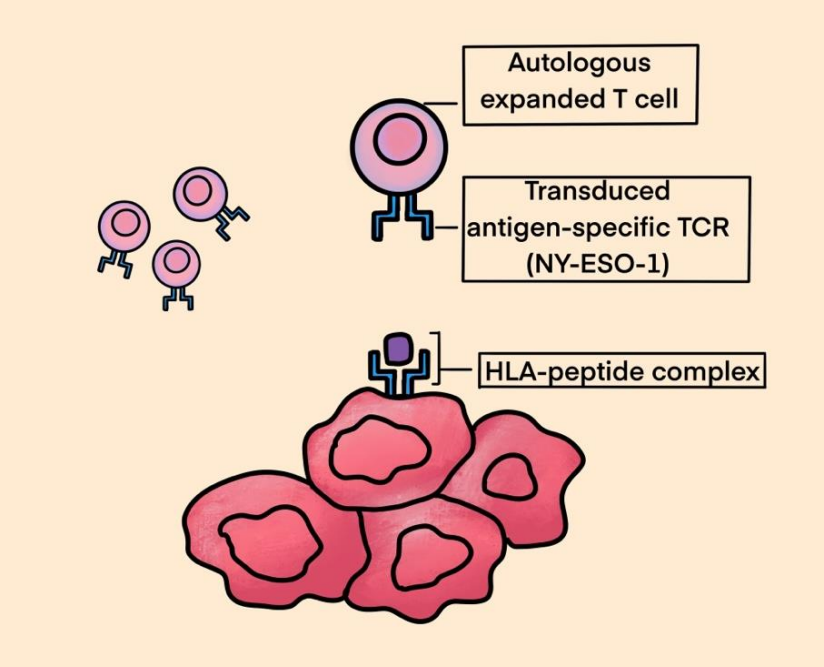

Figure 3. TCR.

T-cell receptor-based therapy utilizes engineered T lymphocytes specifically targeted towards surface tumor antigens. T-cell receptor (TCR)-engineered effector cells use a naturally occurring (or minimally modified) TCR, in contrast to CAR T-cell technology which uses an artificial receptor introduced into the immune effector cells to recognize tumor cell surface proteins[89]. In this strategy, the patient's autologous T-cells are extracted through leukapheresis or from tumor tissue, modified ex-vivo through a lentivirus or retrovirus vector encoding a specific TCR gene, and expanded prior to reinfusion of cells into the patient. TCR recognizes fragments of tumor specific antigens which are presented by MHC molecules on tumor cell surface. The binding of TCR to the MHC-antigen

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complex, in combination with other co-stimulator signals, leads to the activation of T lymphocytes. T-cells can kill tumor directly and attract additional immune cells, thereby eliciting an endogenous immune response. It is prudent to identify tumor specific antigens that are overexpressed in the solid tumors with absent or limited expression in normal tissues. Expression of cancer testis antigens (CTAs), including melanoma antigen gene (MAGE), New York esophageal squamous cell carcinoma gene-1 (NY-ESO-1) and synovial sarcoma X (SSX), is restricted to the germline in normal tissue, but these molecules are broadly upregulated in various tumors. Expression of either NY-ESO-1 and/or MAGE-A4 has been observed in greater than 50% of primary synovial sarcoma specimens. It has also been observed in myxoid liposarcoma, osteosarcomas, pleomorphic liposarcoma and chondrosarcomas, making them appealing targets for TCR based therapies[90–92].

In an interesting phase I/II study by Ramachandran et al. patients with advanced synovial sarcoma were injected with genetically modified autologous T-cells expressing NY-ESO1-1c259, an anti-NY-ESO specific receptor. Engineered T-cell persistence was determined by qPCR. Serum cytokines were evaluated by immunoassay. Transcriptomic analyses and immunohistochemistry were performed on tumor biopsies from patients before and after T-cell infusion. Responses across cohorts were affected by preconditioning and intra-tumoral NY-ESO-1 expression. Of the 42 patients that were evaluated, 1 patient achieved a complete response, 14 achieved partial responses, 24 showed stable disease (SD), and progressive disease (PD) was observed in only 3 patients. The study concluded that a lymphodepletion regimen containing high doses of fludarabine and cyclophosphamide is necessary for genetically modified autologous T-cell persistence and efficacy[93]. Another important pilot trial by Robbins et al. tested autologous TCR-transduced T-cells following lymphodepleting chemotherapy on patients with metastatic synovial cell sarcoma or melanoma expressing NY-ESO-1 that were refractory to standard. Out of 18 patients with NY-ESO-1 positive synovial cell sarcomas, 11 demonstrated objective clinical responses. The estimated overall 3- and 5-year survival rates for patients with synovial cell sarcoma were 38% and 14% respectively [94].

In a phase 2 open label trial called SPEARHEAD 1, D'Angelo et al. aimed to evaluate the efficacy, safety, and tolerability of afamitresgene autoleucel in patients with advanced/metastatic synovial sarcoma (SS) or Myxoid/Round Cell Liposarcoma (MLS). Afamitresgene autoleucel is a genetically engineered autologous specific peptide enhanced affinity receptor (SPEAR) targeting MAGE-A4. Patients with MAGE-A4-expressing tumors underwent leukapheresis for collection of autologous T-cells for processing and manufacture into afamitresgene autoleucel cells, which were infused back into the patients after lymphodepleting chemotherapy. Among 25 evaluable subjects (23 SS and 2 MLS), there were 2 CR, 8 PR and 11 SD (DCR 84%). Side-effects were manageable with mainly low-grade cytokine release syndrome (CRS) and reversible hematologic toxicities due to lymphodepleting chemotherapy[95]. Unlike TCRs, which can only recognize Major Histocompatibility complex (MHC1) restricted peptides, CAR T-cells can target any protein expressed on the surface of tumor cells.

Table 5. Ongoing Trials for TCR therapy in Sarcoma.

Clinical trial	Phase	Intervention	Disease
NCT03462316	Phase I	Anti-NY-ESO-1 (TCR Affinity Enhancing Specific T cell Therapy)	Advanced bone and soft tissue sarcoma that failed first line
NCT05296564	Phase I	Anti-HBI 0201-ESO TCRT (anti-NY-ESO-1 TCR-Gene Engineered Lymphocytes)	NY-ESO-1 -Expressing Metastatic cancers (synovial sarcoma, STS, etc) that failed first line or second line, recurrence of disease, progression of disease
NCT03132922	Phase I	Genetically Engineered Anti-MAGE-A4	MAGE-A4 Positive Tumors (synovial sarcoma, myxoid round cell liposarcoma) failed first line of therapy

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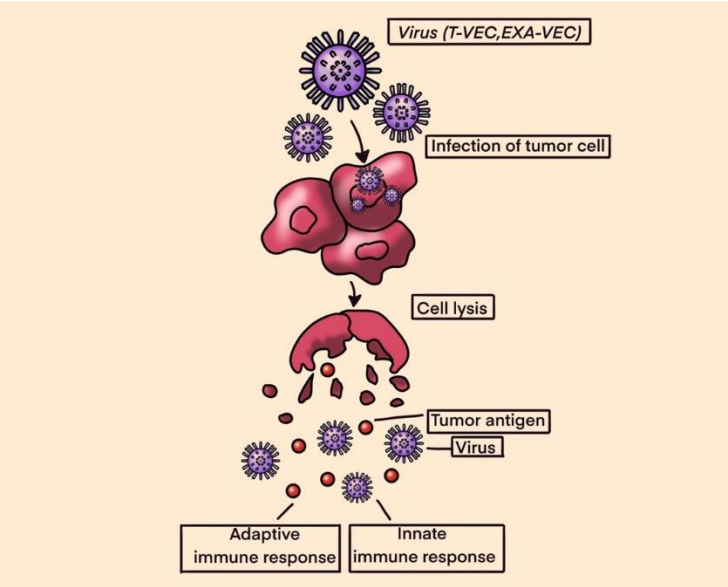

Figure 4. ONCOLYTIC VIRUSES.

Oncolytic viruses are thought to mediate antitumor activity through two distinct mechanisms of action: selective replication within neoplastic cells, resulting in a direct lytic effect on tumor cells; and induction of systemic antitumor immunity. Lysis of tumor cells releases tumor-specific antigens that trigger both the innate and adaptive immune systems [96]. Tumor antigens released by destroyed cancer cells are processed by antigen presenting cells (APC) and presented to the CD4+ and CD8+ lymphocytes, triggering the immune response that enhance tumor destruction. OVs can be divided into two major categories: natural viruses and genetically modified virus strains. Natural viruses include wild type and naturally variant strains of weak viruses[97]. With the development of molecular biology techniques, genetic editing technology is used to optimize these wild virus strains, to weaken viral pathogenicity and improve immunogenicity. Insertion of an exogenous therapeutic gene into the OV genome, to increase its expression in the tumor, makes it is possible to avoid the occurrence of a systemic immune response and enhances the lethality of the virus[98].

In a study by Le Boeuf et al. four oncolytic viruses, reovirus, vaccinia virus, herpes-simplex virus and two rhabdoviruses (vesicular stomatitis virus and maraba virus MG1) were screened for their ability to infect and kill sarcoma cell lines in-vitro. In the in-vitro setting, both rhabdoviruses demonstrated a high potency in their ability to kill sarcoma cells, with - MG1 showing productive viral replication in 18 of 21 tumor samples (86%) and inducing >50% cell death at lower concentrations. Ex vivo, the efficacy of MG1 was tested on murine models infected with tumor cells that were seeded subcutaneously in mice. MG1 was then administered intra-tumorally. Results showed that MG1 effectively replicates in murine sarcoma tumors, leading to eradication of 80% of tumors. Additionally, MG1 also induced the generation of a memory immune response that provided protection against a subsequent tumor challenge[99].

The modified herpes simplex virus known as Talimogene laherparepvec (T-VEC) was approved by the FDA for the treatment of melanoma in 2015. The success of TVEC in melanoma has led to its evaluation in other solid malignancies. In a phase IB/II trial, Monga et al. explored a novel combination of TVEC with external beam radiation therapy (EBRT) administered preoperatively in patients with locally advanced STS of the extremities and trunk. The combination was safe and well-tolerated, however only 5 of the 23 evaluable achieved the primary endpoint of pathological complete response (pCR defined as ≥95% tumor necrosis)[100]. In another phase 2 clinical trial by Kelly et al., treatment with T-VEC plus pembrolizumab was associated with antitumor activity in advanced sarcoma across a range of sarcoma histologic subtypes, with a manageable safety profile. The study met its primary end point, with an ORR at 24 weeks of 35% (95% CI, 15%-59%; n = 7)[101].

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Overall, the aforementioned studies suggest OVs could be promising immunotherapies for the treatment of sarcoma. So far, OVs have had limited success as monotherapies suggesting that OVs will likely require use in combination with other modalities that can overcome known resistance mechanisms, including innate antiviral responses and immunological resistance.

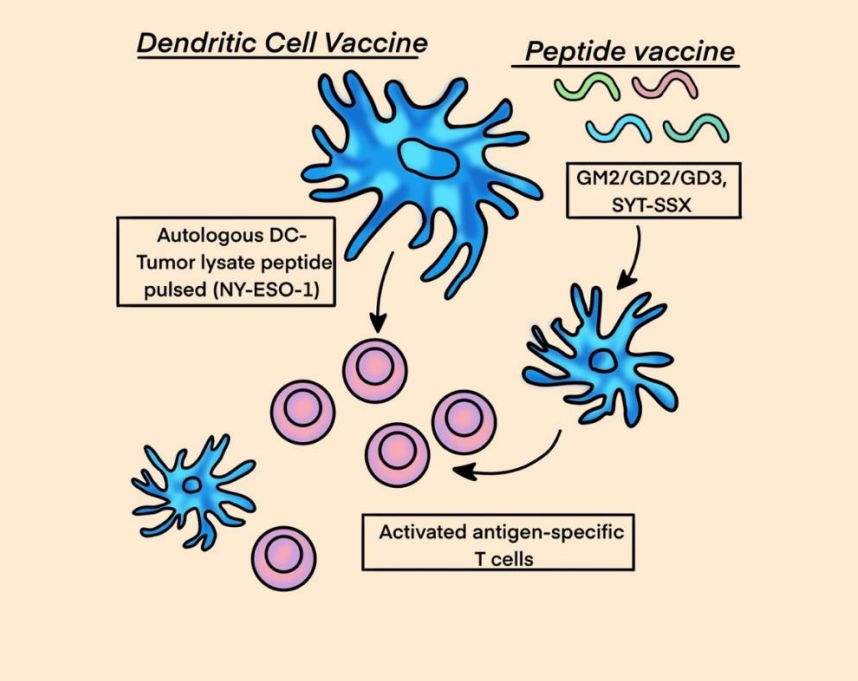

Figure 5. CANCER VACCINES.

Cancer vaccines is a realm of immunotherapy where selected tumor antigens are exogenously administered along with adjuvants/immunostimulants, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or interferon-gamma a to induce the activity of APCs, mainly dendritic cells, aiming to stimulate the adaptive immune system against cancer cells. Antigens for vaccines can be procured from: 1. killed tumor cells, 2. antigens purified from patients with tumor, 3. antigens produced in vitro. The main over-expressed CTAs in sarcomas are NY-ESO-1, MAGE, PRAME (preferentially expressed antigen of Melanoma), BAGE (B melanoma antigen), CAGE (Cancer associated antigen gene); all of them may be excellent candidates for vaccines and as targets for genetically modified adoptive T cells.

In a randomized phase II study by Carvajal et al. immunological adjuvant with a conjugated ganglioside vaccine targeting ganglioside monosialic (GM2), diasialoganglioside (GD2), GD3 and control was tested in patients with metastatic sarcoma following complete metastasectomy. Patients received total of ten injections and imaging was performed to evaluate response. The primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS) and serologic response. Median PFS and 1-year PFS rate were 6.4 months and 35%, respectively, with no difference between arms. The 1-year OS rate was >90%. Serologic responses (IgM and/or IgG) to GM2 and GD2 were observed in 98% and 21% of pts treated with complete vaccine and control, respectively. At weeks 40-68, induction of high (>160) IgM and IgG titers was observed in 52% and 24% of pts receiving vaccine and 0% and 2% of pts receiving control. No difference in PFS was observed between arms [102].

Unique chromosomal translocation events are ubiquitous within certain sarcoma subtypes such as the t(X;18)(p11;q11) translocation in synovial sarcoma or the t(12;16)(q13;p11) translocation in myxoid/round cell liposarcoma, and are attractive vaccine targets as the newly-formed peptide will potentially represent a tumor-specific neoantigen. A fragment of the SYT-SSX fusion peptide that results from the characteristic synovial sarcoma translocation was studied by Kawaguchi et al. as a vaccine in 21 patients with advanced synovial sarcoma that were deemed unresectable and

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previously failed first line of treatment. One out of nine patients administered with the peptide fragment alone did not have disease progression within the study period, and 6 out of 12 patients who received the peptide with an adjuvant and interferon-α had stable disease, one patient exhibited transient shrinkage of a metastatic lesion [103].

In addition to vaccines based on specific peptides, a potential approach to induce tumor recognition is the production of vaccines derived from whole tumor cells combined with immune-enhancing adjuvants (such as IFN-γ and GM-CSF). In a phase II study, Chawla et al. studied CMB305 and atezolizumab compared with atezolizumab alone in soft tissue sarcomas expressing NY-ESO-1[104]. CMB305 is a heterologous prime-boost vaccination regimen created to prime NY-ESO-1-specific CD8 T-cell populations and then activate the immune response with a potent toll-like receptor 4(TLR-4) agonist. Patients with locally advanced, relapsed, or metastatic synovial sarcoma or myxoid liposarcoma were randomly assigned to receive CMB305 with atezolizumab or atezolizumab alone. PFS was 2.6 months and 1.6 months in the combination and control arms, respectively (hazard ratio, 0.9; 95% CI, 0.6 to 1.3). Median OS was 18 months in both treatment arms. The combination of CMB305 and atezolizumab did not result in significant increases in PFS or OS compared with atezolizumab alone. Some patients demonstrated evidence of an anti-NY-ESO-1 immune response and appeared to fare better by imaging than those without such an immune response, however this combination approach merits further evaluation.

Although cancer vaccines for sarcoma appear to be safe and result in an immunological response in most of the patients, limited improvement in clinical outcome of patients suggests that many modifications need to be made to attain better therapeutic outcomes. Further research in this field is warranted.

10. Cancer targeted antibodies

Gangliosides are plasma membrane-bound glycosphingolipids which interact with membrane proteins to regulate the cell signaling pathway [105–107]. The monosaccharide component protruding outside of the cell membrane has antigenic properties and participates in intercellular communication and adhesion [107–109]. Multiple subtypes of gangliosides such as GM3, GM2, GM1 are found on normal cells and regulate the function of membrane bound signaling proteins [109,110]. However, disialoganglioside (GD2) is expressed mostly on tumor cells, with limited expression on normal central and peripheral nerve fibers, mesenchymal stem cells, melanocytes, and lymphocytes [111,112]. This specific tumor antigenic quality of GD2 becomes not only an interesting target in cancer immunotherapy but also a biomarker to predict prognosis and a cancer imaging modality via radioimmunodetection [113,114].

GD2 expression is notable in Ewing sarcoma, usually confirmed by immunostaining [115,116]. In osteosarcoma, the higher intensity of IHC staining was observed in recurrent or relapsed disease tissue section compared to the initial tissue resection [117]. Combination therapy of an anti-GD2 mAb (14G2a) and cisplatin has synergistic effect on apoptosis of the osteosarcoma cells in vitro [118]. In the study, 70-85% of cells apoptosis was observed in osteosarcoma cells treated with cisplatin and 14G2a combination. In soft tissue sarcoma, the expression of GD2 varies from 25% to 93% among different subtypes [119,120].

Another interesting target is CD47, a transmembrane bound protein highly expressed on some tumor cells including angiosarcomas. By producing CD47, tumor cells resist phagocytosis by macrophages; as such, inhibiting CD47 could result in increased tumor cell death [121]. In one of the vitro studies, anti-CD47 therapy increased the production of pro-inflammatory cytokines in the TME of soft tissue sarcomas [122]. In an vivo study using a murine model, the combination of the anti-GD2 antibody dinutuximab and an anti-CD47 antibody (B6H12) was shown to have synergistic activity [123]. In this study, mice with osteosarcoma with pulmonary metastases were treated with a control antibody, anti-GD2, anti-CD47, or a combination of both anti-GD2 and anti-CD47. It was found that anti-GD2 antibody alone did not alter the burden of pulmonary metastases, the anti-CD47 antibody alone reduced the burden of metastases, and the combination treatment eradicated nearly all pulmonary metastatic disease [123]. This is in keeping with a previous trial where dinutuximab (anti-

GD2) was used as a single agent in relapsed osteosarcoma in children and young adults, in which disease control rate did not improve [124]. The reasoning for the combination being more potent is a synergism where anti-GD2 primes tumor cells for phagocytosis via upregulation of surface proteins, while anti-CD47 prevents the tumor's "don't eat me" signals [123]. The ongoing phase I clinical trial (NCT04751383) is testing the combination therapy of magrolimab (anti-CD47) and dinutuximab (anti-GD2) in patients with relapsed or refractory neuroblastoma or relapsed osteosarcoma.

11. Conclusions

The impact of modern immunotherapeutic modalities across various cancer types presents an exciting opportunity for further studies in treatment of sarcomas. An accumulating understanding of the immune microenvironment and antigenic signatures of various sarcoma subtypes has generated promising new targets for immunotherapy. Despite of unrivaled progress in the field of immune oncology over the last decade, early experiences of immunotherapy with sarcomas has been disappointing due to antigenic heterogeneity and rarity of the disease. Although it is difficult to adequately capture the complexity of sarcomas, it appears combination therapies involving ICBs is likely the path forward. When it comes to sarcomas, there is no "one size fits all" strategy and each subtype will require stringent characterization of its immune components and antigenic signatures to select an optimal treatment modality. Further studies are encouraged to develop effective immunotherapy-based regimens for treatment of sarcomas to have better responses and clinical outcomes with manageable toxicity profiles.

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