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REVIEW

Immune checkpoint inhibitors in sarcomas: a systematic review

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Sarcomas are tumors that originate from mesenchymal cells. The variety of sarcomas' response to chemotherapy and the wide range of prognosis reflect their heterogeneity. In order to improve the rates of response, the research has been orientated toward other forms of therapy, such as targeted therapies and immunotherapy or toward combinations of them. Immune checkpoint inhibitors (ICIs) have been the highlight of immunotherapy in the last decade. Although ICIs are already included in the guidelines of different malignancies, their clinical benefit in sarcomas is still under study. Alveolar soft part sarcomas, undifferentiated pleomorphic sarcomas and other subtypes of sarcoma with high presence of tertiary lymphoid structures tend to respond to ICIs, but further investigation is still needed. Furthermore, the search of predictive biomarkers to determine the type of sarcomas that are sensitive to ICIs is still very challenging. This review will focus on the results of clinical trials, which examine the effect of ICIs and their combination with chemotherapy, targeted therapies and other forms of immunotherapy in sarcomas.

Key words: Sarcomas, Immunotherapy, Immune checkpoint inhibitors (ICIs), Programmed death-ligand 1 (PD-L1), Predictive biomarkers

INTRODUCTION

Sarcomas are rare mesenchymal tumors with wide heterogeneity, but with great significance because of the most frequent prevalence in adolescents and young adults. The gold standard of treatment for localized tumors is surgical resection with or without neo/adjuvant chemotherapy and/or radiotherapy based on the exact histotype. Cytotoxic chemotherapy, targeted therapies and immunotherapy have been tested on histologically distinct types of sarcomas. Despite the intense research, sarcomas remain poorly controlled malignancies with a 5-year survival rate reaching 65% for soft tissue sarcomas (STS).[1] These findings highlight the unmet medical need for new treatment strategies.

As far as immunotherapy is concerned, most of the immune checkpoint inhibitors (ICIs) target the binding between either programmed death-ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and B7 protein, allowing immune system to assault tumor cells. These phenomena take place at the lymphoid system and the microenvironment of the tumor. It is widely known that the use of ICIs has changed the therapeutic strategy in many cancer types, such as melanoma, non-small-cell lung cancer, renal cell cancer and head and neck cancer.[2-5] However, not all types of cancer have the same composition of microenvironment, while some types of sarcomas are characterized as non-immunogenic with 'cold' microenvironment or immunologically 'silent', with low tumor mutational burden (TMB) and PD-L1 expression.[6,7]

Immune cells, such as follicular dendritic cells and B cells, tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs), as well as the expression of PD-L1 range between the subtypes of sarcomas and reflect different immunogenetic background of each histotype. More specifically, PD-L1 expression was observed in half of undifferentiated pleomorphic sarcoma (UPS) cases, chondrosarcoma (CS) cases and liposarcoma (LPS) cases, but only in one-third of leiomyosarcoma (LMS) cases in different studies.[8,9] On the contrary, responses to ICIs have been observed in patients with PD-L1-negative sarcomas, such as in a small percentage of synovial sarcomas (SS), thus indicating that PD-L1 is not the ideal predictive biomarker.[10,11]

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This systematic review will focus on results from clinical trials using ICIs in different sarcoma types either as monotherapy or in combination with other modalities.

MATERIALS AND METHODS

A systematic literature search was conducted using PubMed, Medline and ClinicalTrials.gov for the period from 1 January 2012 up to 15 September 2023. The search was reported as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We used the following keywords: sarcoma AND immunotherapy OR sarcoma AND immune checkpoint inhibitors. Language restrictions were applied (only articles in English were considered eligible). Meta-analyses, systematic reviews, prospective and retrospective studies and clinical trials were included. We excluded case reports and *in vitro* studies. Finally, based on the literature and the subject matter, we also involved abstracts, which were presented in the European Society for Medical Oncology (ESMO) and ESMO Immuno-Oncology and American Society for Clinical Oncology (ASCO) congresses.

More specifically, under the search terms 'sarcoma and immunotherapy', we identified 92 publications, while the search of terms 'sarcoma and immune checkpoint inhibitors' revealed 64 publications. Eighty of these 156 publications were considered to be outside of the scope of the present review as they did not refer either to patients with a diagnosis of sarcoma or to the subtypes of sarcomas and were considered irrelevant. Additionally, in case a trial was included in separate publications as duplicated records (e.g. abstract on congress and full text), the most recent manuscript was used and 36 records were further excluded. From the remaining 40 publications, 20 focused on forms of immunotherapy other than ICIs and were also excluded. Finally, 20 abstracts presented in ESMO, ESMO Immuno-Oncology and ASCO congresses and 3 more trials based on the content were included.

RESULTS

Altogether, 43 studies (40 clinical trials—5 phase I, 9 phase I/II and 26 phase II, 1 retrospective study and 2 meta-analyses) were included in our final analysis. In Figure 1, we summarize the elimination process of the initially identified publications. Finally, Table 1 outlines the clinical trials studying ICIs in sarcomas, while a full list of them is added as Supplementary Material, available at https://doi.org/10.1016/j.iotech.2023.100407.

Nivolumab

Nivolumab is one of the first PD-1 ICIs that has been tested as treatment in many solid tumors. It is a human immunoglobulin G4 (IgG4) monoclonal antibody, which attaches to PD-1 and blocks its binding to PD-L1.

Nivolumab has been investigated in different types of sarcomas, either as monotherapy or in combination.

In a phase II trial based on Japanese patients with uterine cervical cancer, uterine corpus cancer or STS, who received nivolumab as monotherapy, the results were disappointing for the STS group—including LPS, LMS, UPS, myxofibrosarcoma and angiosarcoma—with a median progression-free survival (PFS) of 1.4 months.[12] Similar results were shown in a phase II trial, which investigated the clinical activity of nivolumab in metastatic uterine LMS, while the median overall survival (OS) was not met.[13] In the ADVL 1412 phase I/II pediatric and young adults' trial, nivolumab was administered to 85 patients with recurrent or refractory solid tumor, 40 of them with sarcoma, at a dose of 3 mg/kg every 14 days. Young patients with solid tumor did not respond to nivolumab, while the ones with lymphomas showed lower objective responses than in other

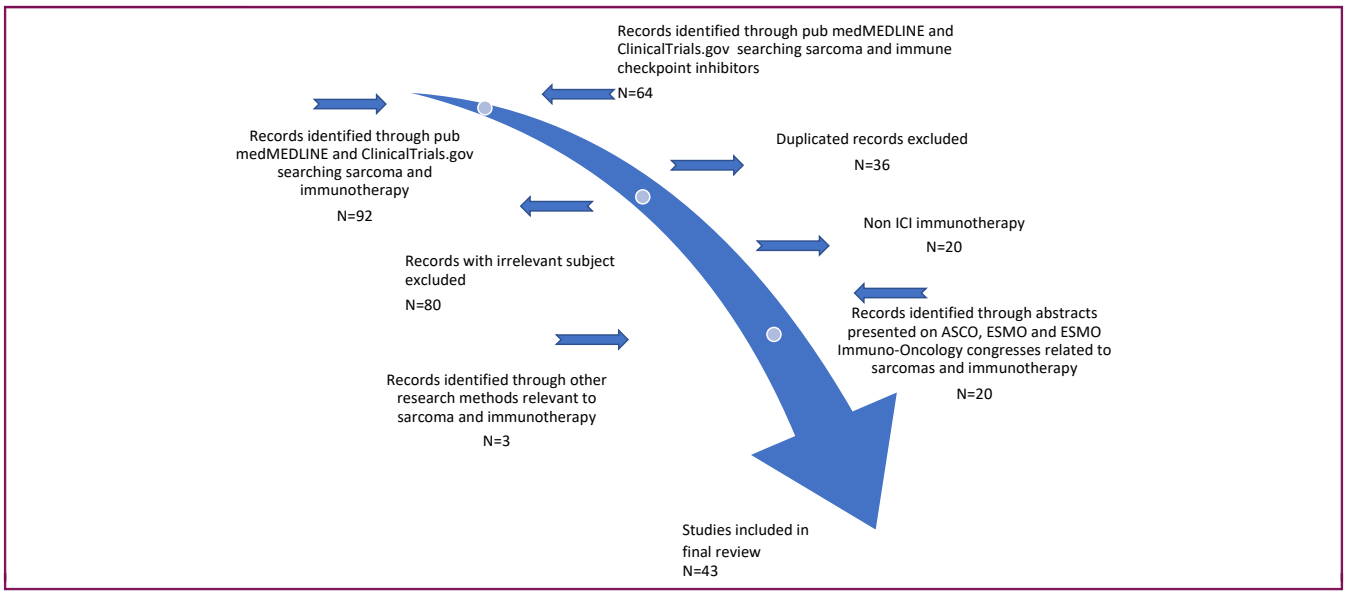

Figure 1. Elimination process of the initially identified publications.
ASCO, American Society for Clinical Oncology; ESMO, European Society for Medical Oncology; ICI, Immune checkpoint inhibitor.

Table 1. Clinical trials and meta-analyses that investigated ICIs in sarcomas

Study type	Name of the trial	Phase	Patient number	Type of tumors	Regimens	mPFS	mOS	Reference
Non-randomized	NCT03013127 PROMO	II	12	Advanced OST	Pembrolizumab 200 mg every 3 weeks	1.7 months	6.6 months	[18]
Non-randomized	NCT02301039 SARC028	II	84, 42 patients on each arm (BS and STS)	Advanced BS and STS (LMS, LPS,UPS, SS, ES, OST, CS)	Pembrolizumab 200 mg every 3 weeks	18 weeks for STS, 8 weeks for BS—ORR 18% in the STS and 5% in the BS	49 weeks for STS and 52 weeks for BS 11.4 months	[16]
Non-randomized	NCT02332668 Keynote-051	I/II	127 with solid tumor, 33 with sarcoma	Melanoma, lymphoma and solid tumor (OST, non-rhabdomyosarcoma STS, embryonal and alveolar rhabdomyosarcoma, inflammatory myofibroblastic tumor, malignant rhabdoid tumor)	Pembrolizumab 2 mg/kg every 3 weeks	1.9 months for other tumor types (except for lymphomas)	9 months for other tumor types (except for lymphomas)	[19]
Non-randomized	NCT03012620 AcSe	II	97	Chordoma, ASPS, DSRCT, SMARCA4-deficient sarcoma or malignant rhabdoid tumor, epithelioid sarcoma, dendritic and clear-cell sarcoma, myxoid liposarcoma and other sarcomas	Pembrolizumab 200 mg every 3 weeks for a maximum of 2 years	2.8 months (6.1 months for chordoma, 6.6 months for ASPS, 2.0 months for SMARCA4-deficient sarcoma or malignant rhabdoid tumor and DSRCT, 2.3 months for epithelioid sarcoma and 2.1 months for other sarcomas)	19.7 months (not reached for chordoma and ASPS, 7.4 months for DSRCT, 2.8 months for SMARCA4-deficient sarcoma, 2.7 months for epithelioid sarcoma and 7 months for other sarcomas)	[17]
Non-randomized	NCT02888665	I/II	37	Advanced anthracycline-naive STS (ASPS, CS, EHE, epithelioid sarcoma, angiosarcoma, LPS, LMS, UPS, rhabdomyosarcoma, myxofibrosarcoma, spindle cell sarcoma, endometrial stromal sarcoma)	Pembrolizumab 200 mg every 3 weeks for 2 years + doxorubicin 45 mg/m^2 or 75 mg/m^2 for 6 cycles from cycle 2	8.1 months, ORR 19%	27.6 months	[45]
Non-randomized	NCT02406781 PEMBROSARC	II	50	Four cohorts: UPS, LMS, other sarcomas, GIST	Pembrolizumab 200 mg every 3 weeks with metronomic cyclophosphamide 50 mg twice daily one week on and one week off	1.4 months	9.2 months for LMS, 5.6 months for UPS, 7.1 months for sarcomas and not reached for GIST	[42]
Non-randomized	NCT02406781 PEMBROSARC	II	15	Advanced OST	Pembrolizumab 200 mg every 3 weeks with metronomic cyclophosphamide 50 mg twice daily one week on and one week off	1.4 months	5.6 months	[43]
Non-randomized	NCT03069378	II	20	Locally advanced or metastatic sarcoma (LMS, UPS, angiosarcoma, undifferentiated and other sarcomas)	Pembrolizumab 200 mg + T-VEC (first dose, $\leq$4 ml $\times$ 10^6 PFU/ml; second and subsequent doses $\leq$4 ml $\times$ 10^8 PFU/ml) every 3 weeks	17.1 weeks ORR 35%	74.7 weeks (disease-specific)	[66]
Non-randomized	NCT02636725	II	33, 12 with ASPS	Advanced sarcoma (LMS, UPS, LPS, myxofibrosarcoma, angiosarcoma and other sarcomas)	Axitinib 5 mg orally twice daily + pembrolizumab 200 mg every 3 weeks for up to 2 years	3 months 65.6%, 4.7 months	18.7 months	[58]
	NCT03056001	II	30			6.9 months	15 months	[46]

Continued

Table 1. Continued

Study type	Name of the trial	Phase	Patient number	Type of tumors	Regimens	mPFS	mOS	Reference
Non-randomized				Unresectable or metastatic STS (LMS, LPS and other sarcomas) and no prior anthracycline therapy	Pembrolizumab 200 mg + doxorubicin 60 mg/m^2 (75 mg/m^2 dose escalation as per investigator's discretion) every 3 weeks			
Non-randomized	NCT03414229	II	29	Advanced sarcoma (LPS, UPS, myxofibrosarcoma, EHE, angiosarcoma and other sarcomas)	Pembrolizumab 200 mg every 3 weeks+ epacadostat 100 mg twice daily	8 months	Not estimated, 85.2% at 24 weeks	61
Non-randomized	NCT03899805	II	19	STS, results from LMS cohort	Pembrolizumab 200 mg + eribulin 1.4 mg/m^2 (day 1, 8) every 3 weeks	11.1 weeks	Not reached	62
Non-randomized	NCT03123276	I	13	Advanced LMS and UPS	Pembrolizumab 200 mg + gemcitabine 800 mg/m^2 or 1000 mg/m^2 or 1200 mg/m^2 (day 1, 8 for 6-8 cycles)	5.1 months	—	47
Non-randomized	NCT04577014	I/II	13	Naive unresectable or metastatic high-grade STS (LMS, UPS, dd or pleomorphic LPS, angiosarcoma and myxofibrosarcoma)	Gemcitabine 900 mg/m^2 on day 1, 8 + docetaxel 75 mg/m^2 on day 8 every 3 weeks (6 cycles of chemotherapy) with retifanlimab 210 mg or 375 mg on day 1 from the second cycle until PD	60% and 44% at 24 weeks (in the run-in and de-escalation cohorts, respectively)	—	52
Non-randomized	NCT04356872	II	24	Advanced STS (UPS, SS, myxoid LPS and dd LPS)	Sintilimab 200 mg + doxorubicin 60 mg/m^2 on day 1 + ifosfamide 1.8 g/m^2/day on days 1-5 every 3 weeks for up to 6 cycles, followed by sintilimab until PD	ORR 62.5%	—	53
Non-randomized pediatric	NCT02304458 ADVL 1412	I/II	85, 40 patients with sarcoma	Recurrent or refractory non-CNS solid tumor Sarcoma subtypes: epithelioid, Ewing's, OST, rhabdomyosarcoma, undifferentiated and other	Nivolumab 3 mg/kg every 14 days	No objective response in non-hematological tumors	—	14
Non-randomized	NCT02428192	II	12	Metastatic uterine LMS	Nivolumab 3 mg/kg every 2 weeks	1.8 months	Not met	13
Non-randomized	JapicCTI-163212	II	21	Uterine cervical tumor, uterine corpus cancer or STS (LPS, LMS, UPS, myofibrosarcoma, angiosarcoma)	Nivolumab 240 mg every 2 weeks	1.4 months	Not estimable	12
Non-randomized	NCT00140855	II	6	Advanced SS	Ipilimumab 3 mg/kg every 3 weeks for 3 doses	1.85 months Response rate 0%	8.75 months	21 Early termination
Non-randomized pediatric	NCT01445379	I	33, 17 with sarcoma	Recurrent or refractory solid tumor Sarcoma subtypes: OST, SS, rhabdomyosarcoma, clear cell, pleomorphic and undifferentiated	Ipilimumab 5 mg/kg for <12-year-old patients and 10 mg/kg for 12-21-year-old patients every 3 weeks for the first 4 doses and then every 12 weeks	18% SD (6 of 33), no PR or CR		20
Randomized non-comparative	NCT02500797 Alliance A091401	II	84	Locally advanced, unresectable, or metastatic sarcoma (angiosarcoma, BS, LPS, UPS, LMS, spindle cell sarcoma, SS and other)	Nivolumab 3 mg/kg every 2 weeks or nivolumab 3 mg/kg + ipilimumab 1 mg/kg every 3 weeks for 4 doses and then nivolumab 3 mg/kg alone every 2 weeks	1.7 months in the monotherapy group, 4.1 months in the combination group	10.7 months in the monotherapy group, 14.3 months in the combination group	15

Continued

Table 1. Continued

Study type	Name of the trial	Phase	Patient number	Type of tumors	Regimens	mPFS	mOS	Reference
Non-randomized	NCT02834013; SWOG S1609	II	16	Metastatic or unresectable angiosarcoma	Ipilimumab 1 mg/kg every 6 weeks plus nivolumab 240 mg every 2 weeks	6 months PFS 38%, ORR 25%	Not reached in a follow-up of 12.1 months	[91]
Randomized	NCT02880020	II	29	GIST	Nivolumab 240 mg every 2 weeks ± ipilimumab 1 mg/kg every 6 weeks	In nivolumab arm 8.57 weeks, in the combination arm 9.1 weeks	—	[34]
Randomized non-comparative	NCT03307616	II	24 (preliminary results)	Retroperitoneal dd LPS (arm A, B) and UPS (arm C, D)	Arm A: neoadjuvant nivolumab 3 mg/kg every 2 weeks, arm B: neoadjuvant ipilimumab 3 mg/kg+ nivolumab 1 mg/kg followed by 2 doses of nivolumab 3 mg/kg every 2 weeks, arm C: neoadjuvant 1 dose of nivolumab 3 mg/kg followed by nivolumab 3 doses, 3 mg/kg every 2 weeks + radiotherapy, arm D: 1 dose nivolumab 1 mg/kg+ ipilimumab 3 mg/kg, followed by nivolumab 3 doses, 3 mg/kg every 2 weeks + radiotherapy	Not reached, for resectable UPS and 18 months for resectable, largely recurrent, retroperitoneal dd LPS	Not reached, 2-year overall survival rate was 90% for UPS and 82% for dd LPS	[36-38]
Non-randomized	NCT03277924 IMMUNOSARC	Ib/II	68: 16 in phase Ib and 52 in phase II	Advanced and progressing STS (OST, SS, UPS, ASPS, angiosarcoma, CS, EHE, clear-cell, solitary fibrous, epithelioid sarcoma)	Sunitinib 37.5 mg as induction and then 25 mg + nivolumab 3 mg/kg on day 15 and every 2 weeks	5.6 and 6 months (central and local assessment)	24 months	[55]
Non-randomized	NCT03277924 IMMUNOSARC	II	40	BS cohort (OST, CS, Ewing's sarcoma, bone UPS)	Sunitinib 37.5 mg as induction and then 25 mg + nivolumab 3 mg/kg on day 15 and every 2 weeks	3.7 months	14.2 months	[92]
Retrospective	—	—	28	Metastatic or locally advanced STS (LMS, UPS, SS, dd LPS, epithelioid or intimal sarcoma, dermatofibrosarcoma protuberans, malignant peripheral nerve sheet tumor, DSRCT) or BS (OST, CS)	Nivolumab 3 mg/kg every 2 weeks ± pazopanib 400-800 mg daily	Response: 3 PR, 9 SD, 50% clinical benefit	—	[54] Withdrawn
Non-randomized	NCT03190174	I/II	9 in phase I	Advanced UPS, LPS, OST, CS, ES	Nivolumab 240 mg every 3 weeks and nab-sirolimus (ABI-009) 100 mg/m^2 at day 8 and 15 starting from the second cycle	Not reached in the preliminary results	—	[60]
Non-randomized	NCT03282344	I/II	50	LMS, UPS, dd LPS, CS, OST, AS, ASPS, SS/small blue round cell and other	NKTR-214 0.006 mg/kg + nivolumab 360 mg every 3 weeks	NA (ORR 8%)	NA	[63]
Non-randomized	NCT03590210; NiTraSarc	II	92; 55 in group A and 37 in group B	Group A: LPS, LMS, group B: non-L-sarcoma (UPS/not otherwise specified sarcoma, SS, epithelioid and fibromyxoid sarcoma, fibrosarcoma)	3 ['late combination cohort' (LCC)] or 2 ['early combination cohort' (ECC)] cycles of trabectedin 1.5 mg/m^2, followed by the combination of trabectedin 1.5 mg/m^2 + nivolumab 240 mg for up to 16 cycles	6 months PFS 13.9%; 8.7% in LCC and 23.1% in ECC		[48]

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Study type	Name of the trial	Phase	Patient number	Type of tumors	Regimens	mPFS	mOS	Reference
Retrospective analysis		II	20	Advanced STS (UPS, LMS, SS, myxoid LPS, CS)	Trabectedin 1.5 mg/m^2 for 24 h every 3 weeks + nivolumab 3 mg/kg every 2 weeks	>11.6 months	>14.2 months	[49]
Non-randomized	NCT01643278	IB	28	Advanced or metastatic GIST and STS (high-grade, clear-cell and epithelioid sarcoma, LMS, uterine smooth muscle tumor of uncertain malignant potential, malignant peripheral nerve sheath tumor and chordoma)	Dasatinib 140 mg for 7 days, dasatinib + ipilimumab 3 mg/kg once on weeks 1, 4, 7 and 10. Beginning on week 24, dasatinib 140 mg+ ipilimumab 3 mg/kg once every 12 weeks	2.8 months	13.5 months	[59]
Non-randomized pediatric	NCT03141684 ML39345	II	43	ASPS	Atezolizumab 1200 mg in adults or 15 mg/kg (1200 mg max) in pediatric patients	ORR 37.2%		[30]
Randomized	NCT02609984	II	89	Locally advanced, relapsed or metastatic sarcoma (synovial or myxoid/round cell LPS)	CMB305 (LV305 1 × 10^{10} vector genomes) on days 0 and 14 followed by every 2 weeks with G305 at a dose of glucopyranosyl lipid A 5 µg mixed with NY-ESO-1 protein 250 µg Atezolizumab 1200 mg every 3 weeks for 2 years OR atezolizumab 1200 mg every 3 weeks alone	2.6 months for the combination, 1.6 months for atezolizumab alone	18.2 months for the combination, 18 months for atezolizumab alone	[64,65] Terminated
Non-randomized pediatric	NCT02541604 iMATRIX	I/II	90	Solid tumor and lymphoma Subtypes of sarcoma: OST, Ewing's sarcoma, rhabdomyosarcoma, non-rhabdomyosarcoma STS, malignant rhabdoid tumor, atypical teratoid rhabdoid tumor	Atezolizumab 1200 mg every 3 weeks, patients younger than 18 years received dose of 15 mg/kg	Best overall response: 13% SD, 5% PR		[31]
Non-randomized	NCT02836834	I	33	Advanced or refractory cancer, 12 patients with ASPS	Toripalimab 1 mg/kg, 3 mg/kg and 10 mg/kg every 2 weeks	11.1 months for patients with ASPS	34.7 months for patients with ASPS	[33]
Non-randomized	NCT03623581	II	37	Unresectable, recurrent or metastatic ASPS	Geptanolimab (3 mg/kg) every 2 weeks	6.9 months	Immature results	[93]
Non-randomized	NCT02815995	II	57	LPS, LMS, angiosarcoma, UPS, SS, OST, ASPS, chordoma and other sarcomas	Durvalumab 1500 mg + tremelimumab 75 mg every 4 weeks for 4 cycles followed by durvalumab 1500 mg every 4 weeks up to 12 months	4.5 months	20.8 months	[39]
Non-randomized	NCT03085225	IB	40; 9 at STS	Unresectable or metastatic STS and relapsed ovarian carcinoma	Trabectedin 1.2 mg/m^2 + durvalumab 1120 mg every 3 weeks	6-month PFS 28.6%, ORR 7% in STS cohort	—	[51]
Non-randomized	NCT03074318	I/II	33	Advanced LMS and LPS	Trabectedin 1.0 mg/m^2 + avelumab 800 mg	8.3 months		[50]
Non-randomized	NCT03359018	II	43	Advanced OST	Apatinib 500 mg once daily+ camrelizumab 200 mg every 2 weeks	6.2 months	11.3 months	[56]

Continued

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Study type	Name of the trial	Phase	Patient number	Type of tumors	Regimens	mPFS	mOS	Reference
Meta-analysis			88	Metastatic STS (UPS, ASPS, LMS, SS, LPS, angiosarcoma, epithelioid sarcoma, fibroblastic sarcoma, sclerosing epithelioid fibrosarcoma, myxofibrosarcoma and other)	Pembrolizumab or nivolumab or ipilimumab or nivolumab + ipilimumab or cabiralizumab plus nivolumab or other combinations of ICI	4.1 months	19.1 months	[40]
Meta-analysis			1012	STS (UPS, ASPS, LPS, SS, CS, GIST, dd LPS, LMS, OST, uterine LMS, DSRCT, myxoid LPS, Ewing's, SMARCA4-deficient, epithelioid, Kaposi and clear-cell sarcoma, angiosarcoma, chordoma, myxofibrosarcoma, rhabdomyosarcoma and other)	ICIs	1.8-11.1 months	6.1-34.7 months	[41]

ASPS, alveolar soft part sarcoma; BS, bone sarcoma; CNS, central nervous system; CR, complete response; CS, chondrosarcoma; dd, dedifferentiated; DSRCT, desmoplastic small round cell tumor; EHE, epithelioid hemangioendothelioma; ES, Ewing's sarcoma; GIST, gastrointestinal stromal tumor; ICI, immune checkpoint inhibitor; LMS, leiomyosarcoma; LPS, liposarcoma; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NTRK, neurotrophic tyrosine receptor kinase; NY-ESO-1, New York esophageal squamous cell carcinoma-1; ORR, overall response rate; OST, osteosarcoma; PD, progressive disease; PFU, plaque-forming unit; PR, partial response; SD, stable disease; SS, synovial sarcoma; STS, soft tissue sarcoma; T-VEC, talimogene laherparepvec; UPS, undifferentiated pleomorphic sarcoma.

trials.[14] These results come along with the fact that solid tumors in children are correlated with low TMB and immunotherapy is a reasonable treatment, when tumors are linked with germline mutations in mismatch repair genes. Furthermore, nivolumab, as monotherapy, has no efficacy in patients with unresectable or metastatic sarcoma, who received either nivolumab alone or nivolumab plus ipilimumab in the Alliance A091401 phase II trial. Patients who received nivolumab alone had an inferior response rate compared to chemotherapeutic regimens and a median PFS of only 1.7 months. Only 3 of 38 patients in the monotherapy arm had a partial response.[15]

Pembrolizumab

As a humanized monoclonal antibody, pembrolizumab has been tested in almost all cancer types in the research base and has been approved in several of them. It is also an anti-PD-1 monoclonal antibody, which has been explored in several types of sarcomas.

The SARC028 phase II trial enrolled 84 patients equally separated between bone sarcoma (BS) and STS groups. The BS group was further separated into patients with Ewing's sarcoma (ES), osteosarcoma (OST) and CS, while the STS group was separated into patients with UPS, LPS, LMS and SS. All patients received pembrolizumab 200 mg every 21 days. The objective response rate (ORR) was quite high in UPS with one patient attaining complete response and two others partial response while 20% in LPS showed partial response. Disappointingly, ORR in BS was only 5% and none of the patients with ES responded to pembrolizumab. Additionally, median PFS was 30 weeks for UPS and 25 weeks for LPS, but only 8 weeks for BS.[16]

The heterogeneous activity of pembrolizumab monotherapy between different subtypes of sarcomas was also shown in the AcSe trial, a phase II, multicentric study. The recently updated results indicated an ORR of 6.2%, a median PFS of 2.8 months, and a median OS of 19.7 months with heterogeneity of response between different histotypes in favor of alveolar soft part sarcomas (ASPS) and SMARCA4-deficient sarcoma or malignant rhabdoid tumor with 50% and 25% partial response, respectively. One (7%) patient with ASPS and one (17%) with epithelioid sarcoma had complete response.[17] Unfortunately, the results of the PROMO trial with 12 enrolled patients with advanced OST receiving pembrolizumab monotherapy every 3 weeks were so disappointing that the trial was stopped early. No clinical benefit and no correlation of response with PD-L1 expression was observed.[18]

Finally, pembrolizumab was administered at a dose of 2 mg/kg every 21 days to 154 pediatric patients with melanoma, PD-L1-positive solid tumors and lymphoma in a phase I/II trial. Only 7 of 33 patients with sarcoma had a reduction of tumor size and only 3 of them met the criteria for a partial response.[19]

Ipilimumab

Ipilimumab is a monoclonal antibody with anti-CTLA-4 role. By blocking a down-regulative signal in T cells, the immune

system targets tumor cells. As monotherapy, ipilimumab was used in one phase I and one phase II study for sarcomas.[20,21] Based on the phase I pediatric trial, ipilimumab was administered in recurrent or refractory solid tumors; 17 of 33 patients had sarcoma. No objective response was observed and only 18% of the patients had stable disease (SD).[21] Furthermore, only six patients with advanced SS were enrolled in the phase II trial. The study was ended early due to slow accrual and the fact that all patients had a disease progression.[20]

Atezolizumab

Atezolizumab is a humanized IgG1 monoclonal antibody against PD-L1. It is approved as treatment in triple-negative breast cancer, non-small- and small-cell lung cancer, hepatocellular cancer and urothelial cancer.[22-29] Its use in sarcomas has been explored and results are available from two trials. Naqash et al. investigated the effect of atezolizumab in ASPS. The results were promising, as 15 of 43 patients have confirmed response, while a complete response was observed. Besides, 25 of 43 patients had SD with a median duration of response of 16.5 months.[30]

In the iMATRIX phase I/II study, none of the pediatric patients with BS and STS who received atezolizumab had an objective response and only 6 of 40 patients had SD.[31] However, this trial was conducted with many protocol deviations.

Other ICIs

Other ICIs were further investigated in advanced sarcomas. Spartalizumab is a humanized IgG4κ monoclonal antibody which blocks the connection of PD-1 with PD-L1 and PD-L2. Naing et al. studied the efficacy of spartalizumab in patients with advanced or metastatic solid tumors. According to the results, 28% of solid tumors were sarcomas and two of the patients with sarcoma had SD for 2 and 1 year, respectively.[32] Unfortunately, this trial was excluded from our analysis because of the lack of histotypes of sarcomas.

Toripalimab, another recombinant humanized anti-PD-1 monoclonal antibody, has a higher affinity binding to PD-1 than the traditional ICIs nivolumab and pembrolizumab. In a phase I clinical trial, toripalimab was administered to 33 Chinese patients with advanced or refractory tumors; 12 of them had ASPS. These 12 patients had better responses than patients with other solid tumors. More specifically, for these 12 patients, ORR was 25.0%, duration of complete response was 91.7%, median OS was 34.7 months and median PFS was 11.1 months.[33]

Combination of ICIs

Since immunotherapy as monotherapy was ineffective in patients with sarcoma, the combination of two ICIs with different ways of action was inevitable. The most common combination of ICIs to other cancer types is nivolumab and ipilimumab. In the randomized Alliance A091401 trial, the combination of nivolumab plus ipilimumab for metastatic, locally advanced or unresectable sarcomas was more effective than monotherapy with nivolumab and the response rate was estimated at 16%, meeting the predefined primary endpoint of the study. Responses were observed in ASPS, UPS, LMS, myxofibrosarcoma and angiosarcoma with three (8%) partial responses in the nivolumab arm and five (12%) partial responses and two (5%) complete responses in the nivolumab/ipilimumab arm.[15] On the contrary, nivolumab plus ipilimumab did not have a synergistic effect in gastrointestinal stromal tumors (GIST).[34]

In the neoadjuvant setting, ICIs in combination with radiotherapy can create preoperative hyalinization/fibrosis, as well as replace tumor areas with amorphous palely eosinophilic and collagenous material and sparse fibroblasts according to the histotype of sarcomas.[35] This was the aim of a phase II trial of neoadjuvant treatment with nivolumab ± ipilimumab ± radiotherapy in patients with resectable dedifferentiated LPS of the retroperitoneum or UPS.[36] According to preliminary results of this trial, 14 patients with dedifferentiated LPS and 9 with UPS were enrolled and the first cohort showed 22.5% median pathological response, while the UPS cohort showed a very promising 95%.[37] In the 2 years' follow-up, the median PFS was 18 months for patients with resectable retroperitoneal dedifferentiated LPS, while the median PFS was not reached for patients with resectable UPS. The 2-year PFS was 70% for the cohort of patients with UPS and 35% for those with dedifferentiated LPS, while the respective 2-year OS was 90% and 82%, respectively.[38]

The same pattern of response based on the histologic type was observed in a phase II study exploring the combination of durvalumab and tremelimumab in sarcoma. Better responses were observed in ASPS, while worse responses in LPS.[39] Furthermore, a retrospective analysis of 88 patients with metastatic STS, who received ICIs (pembrolizumab, nivolumab, ipilimumab) or a combination of them, indicated UPS and LMS as the histologic types of sarcomas with the greatest response to immunotherapy. Not surprisingly, 28% of patients with UPS and 45% with LMS had a partial response.[40]

In a meta-analysis of all STS treated with different therapeutic plans with anti-PD-1 monotherapy, anti-CTLA-4 monotherapy, combination of them, or combination of ICI with tyrosine kinase inhibitors (TKIs), chemotherapy and immunomodulator, PFS and OS have shown a wide range based on histologic subtype. In ASPS, higher responses were seen for combination with TKIs and anti-CTLA-4.[41] On the contrary, for UPS, best responses were seen for the combination of anti-PD-1 and anti-CTLA-4 with chemotherapy. The ORR in advanced first-line treatment was higher in combination with chemotherapy and those responses vary among different histologic subtypes. Finally, we are expecting the results of various studies with combinations of ICIs in sarcomas, especially of the RAR-Immune study, the only phase III study exploring the efficacy of nivolumab and ipilimumab in sarcomas.

Combination with chemotherapy

A recent trial that examined immunotherapy plus chemotherapy in sarcomas is the phase II PEMBROSARC trial. Fifty patients with advanced tumors were enrolled into four cohorts: GIST, LMS, UPS and other sarcomas, and received pembrolizumab added to metronomic cyclophosphamide. Best response was SD for 16 patients, while progressive disease (PD) was observed in 31 of them. Disappointingly, median PFS was 1.4 months for all cohorts, although median OS was not reached for GIST, 9.2 months for LMS, 5.6 months for UPS and 7.1 months for other sarcomas.[42] The results of the same trial, including only 15 patients with advanced OST, were presented separately. Four out of five patients discontinued because of PD and one out of five because of adverse events. As best response, they observed PD in 8 out of 15 patients, SD in one-third of them and PR only in 6%. There was no correlation of PD-L1 expression and response.[43] Finally, at a recent update of the PEMBROSARC trial, TLS-positive sarcomas demonstrated a 30% ORR in comparison to an ORR of 2.4% in unselected all-comer cohorts. More specifically, the best response was partial response for nine patients (30%), five patients with dedifferentiated LPS, three with epithelioid sarcomas and one with LMS.[44]

Notably, doxorubicin is one of the chemotherapeutic agents with sufficient efficacy in STS. Pollack et al. used doxorubicin plus pembrolizumab in patients with anthracycline-naive STS showing satisfying response of tumor. It is worth noting that the response was durable in half cases of dedifferentiated LPS and in two out of three patients with UPS. The unrelatedness of PD-L1 expression with PFS and OS and the association of the presence of TILs with inferior PFS may mirror an aggressive tumor biology rather than a connection of PD-L1 expression to immunotherapy response.[45] In a study with a similar therapeutic plan, PFS and OS were longer in the combination arm compared to monotherapy with doxorubicin. The results highlight the synergistic efficacy of pembrolizumab and doxorubicin.[46] In addition, other chemotherapy regimens, such as gemcitabine, are combined with pembrolizumab in LMS and UPS. A phase I trial has shown a median PFS of 5.1 months, while the results of the maximum tolerated dose of gemcitabine are ongoing.[47]

Another drug combination that has been examined against sarcoma is nivolumab with trabectedin. Fifty-five patients were enrolled in the L-sarcoma group and 37 in the non-L-sarcoma group of the NiTraSarc trial. They received trabectedin with nivolumab with two ('early combination cohort') or three ('late combination cohort') initial cycles of trabectedin followed by the combination. The preliminary results of 36 patients of the non-L-sarcoma group were demonstrated in the ASCO annual meeting in 2021. After a median follow-up of 5 months, median 6-month PFS rate was 13.9% for all patients, 8.7% in the late and 23.1% in early cohorts. Two of those patients had partial response and 10 had SD.[48] Furthermore, a retrospective analysis with nivolumab and trabectedin in advanced STS demonstrated safety and efficiency of this combination against sarcoma.[49] Wagner et al. combined trabectedin with avelumab against advanced LMS and LPS. ORR as the primary endpoint was not met, but the median PFS was 8.3 months with a 6-month PFS marginally over 50%.[50] A corresponding rate of 28.6% was observed in the TRAMUNE trial, which explored the combination of trabectedin and durvalumab in advanced pretreated STS. One patient with LMS experienced partial response.[51] Finally, two other anti-PD-1 antibodies, sintilimab and retifanlimab, co-administered with chemotherapy have shown an ORR of ~60% in advanced STS.[52,53]

Combination with other targeted therapy and other forms of immunotherapy

Under the same aspect, the dual targeted treatment against sarcoma was expanded in combination of ICIs with targeted therapy or other forms of immunotherapy. The basic category of targeted treatment is TKIs, such as pazopanib, sunitinib, apatinib and axitinib. Although a retrospective trial with 28 patients who received nivolumab alone or nivolumab plus pazopanib has shown 50% clinical benefit, a randomized phase II study was withdrawn because of lack of recruitment.[54] In the phase Ib/II IMMUNOSARC trial, 16 patients with STS were recruited in phase Ib and 52 in phase II, who received nivolumab and sunitinib. ORR was 21%, while the 18-month OS was 100%, 75% and 44% for those patients with response, SD and PD, respectively ($P = 0.01$). Almost half of the patients had a 6-month PFS, while one patient with angiosarcoma experienced complete response, and two patients with ASPS, one with angiosarcoma, one with SS and one with extraskeletal myxoid CS experienced a partial response.[55] In a phase II study for OST, camrelizumab combined with apatinib did not show statistically significant PFS in comparison to monotherapy with apatinib. Patients with only pulmonary metastases and overexpression of PD-L1 showed better PFS.[56]

Axitinib is a TKI selectively against vascular endothelial growth factor (VEGF) receptors 1-3, c-KIT and platelet-derived growth factor receptor. It was added to pembrolizumab against advanced sarcomas. Thirty-three patients were enrolled and 12 of them had ASPS. Despite dual targeting, none of the 33 patients had a complete response, 25% achieved partial response and almost 33% of them had SD. 72.7% of the patients with ASPS showed clinical benefit, due to the translocation of the *ASPSCR1-TFE3* fusion gene, which is observed in this subtype of sarcomas and leads to aberrant transcription of downstream target genes and consequently up-regulates proangiogenic factors including VEGF.[57] On the contrary, PFS was not correlated with PD-L1 positivity or increased TIL score.[58] Another combination of ICIs with TKIs is dasatinib and ipilimumab, which has been used in patients with GIST and other STS. Unfortunately, dasatinib did not increase the efficacy of ipilimumab and did not change the microenvironment in favor of the second one; conclusions were indicated by the median PFS of 2.8 months and the median OS of 13.5 months.[59]

Mammalian target of rapamycin (mTOR) and indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors could act cooperatively with ICIs against sarcoma. Under this theory, ABI-009 or else nab-sirolimus, an mTOR inhibitor, was combined with nivolumab for treating patients with OST, CS, ES, UPS and LPS. Based on the preliminary results of this study, the median PFS has not been reached.[60] Additionally, IDO1 inhibitor epacadostat added to pembrolizumab in different types of sarcomas showed a median PFS of 8 months.[61] On the contrary, the combination of pembrolizumab with eribulin showed a median PFS of 11.1 weeks in LMS.[62] Finally, although the results from a study with the combination of neurotrophic tyrosine receptor kinase inhibitor + nivolumab received by patients with different types of sarcomas are expected, the preliminary results showed an ORR of only 8%.[63]

In several clinical trials, ICIs were combined with other forms of immunotherapy and genetically engineered viruses. CMB305 is a heterologous targeted vaccination regimen priming New York esophageal squamous cell carcinoma 1 (NY-ESO-1)-specific CD8 T-cell populations, in order to activate the immune response with a potent Toll-like receptor 4 agonist. It was given along with atezolizumab in locally advanced, relapsed or metastatic SS or myxoid/round cell LPS. Unfortunately, this trial was terminated due to statistically insignificant prolongation of PFS or OS, but patients with NY-ESO-1 T-cell response showed better OS.[64,65] On the contrary, patients with advanced sarcomas, who were treated with pembrolizumab and talimogene laherparepvec, had similar results compared with the same treatment plan in patients with melanoma. ORR was 35%, while median PFS was 17.1 weeks and disease-specific survival was 74.7 weeks. Two patients with angiosarcoma, two with UPS, one with myxofibrosarcoma, one with epithelioid and one with unclassified sarcoma had a partial response.[66]

BIOMARKERS

PD-L1, as a ligand for the T-cell immune checkpoint receptor PD-1, predicts response to ICIs in many solid tumors, such as lung cancer and melanoma.[67,68] On the contrary, it provides us with controversial results as prognostic and predictive biomarker for treatment with ICIs in many other solid tumors,[69] especially in STS. Different immunohistochemistry antibodies for PD-L1 expression were used in many trials, while RNA sequencing does not reproduce immunohistochemistry results.[70] Although the combination of PD-L1 expression in the tumor and PD1-positive lymphocytes is better associated with prognosis of STS,[8] TMB has shown a much better correlation with response to ICI especially in SS.[10] TMB high values have been demonstrated in UPS, LMS, cardiac sarcomas and SS.[10,70-73]

Tertiary lymphoid structures (TLS) including T cells, follicular dendritic cells and B cells are part of the tumoral microenvironment and seem to have a better prognostic and predictive value.[74] As previously mentioned, TLS- and intratumoral plasma cell-rich STS showed better response to pembrolizumab based on recently updated results of the PEMBROSARC trial.[44] Additionally, tumor-associated antigens, such as NY-ESO-1 and melanoma-associated antigen (MAGE), which are expressed in high-grade STS, could render good candidates for immunotherapy, as they can develop tumor-specific immune responses. Representatively, NY-ESO-1 is expressed in 70%-80% of SS and in 95% of myxoid round cell LPS.[73] Finally, MAGE-A1, MAGE-A3 and NY-ESO-1 vaccinations are under investigation as therapeutic options in different solid tumors, including sarcomas.[75-77]

DISCUSSION

Sarcomas are heterogeneous tumors with many histotypes being resistant to chemotherapy. Consequently, the search for new therapeutic approaches is essential. As immunotherapy with ICIs emerged to change the therapeutic strategy in many solid tumors, there is still an unmet need to elaborate immunotherapy in the therapeutic armamentarium of sarcomas. On the contrary, the rarity of these tumors and the great heterogeneity are major caveats in the effort of conducting clinical trials with sarcoma patients and the reason they have not yet provided us with mature results.

Based on our review, the median PFS of trials offering immunotherapy in sarcoma patients ranged from 1.4 to over 11.2 months in a retrospective analysis and the median OS from 5.6 to 34.7 months. This wide range reflects the heterogeneity of sarcomas and their response to immunotherapy. More specifically, patients with OST had the worst responses to ICI either as monotherapy, dual blockade or in combination with chemotherapy or TKIs.[16,18,43,56,60] On the contrary, patients with ASPS followed by patients with LMS and UPS are more likely to respond to ICI or in combination with TKIs or chemotherapy.[15,33,39-41,57,78] It is worth mentioning that the combination of an anti-CTLA-4 regimen with TKI showed better effectiveness in ASPS than in UPS. Furthermore, the addition of chemotherapy to ICIs might offer benefits in patients with UPS.[41] These results come along with higher TMB and higher number of non-synonymous mutations to these subtypes of sarcomas, in comparison with other histotypes, such as OST.[41,79,80]

The reason that sarcomas are not responsive to immunotherapy compared with other solid tumors, such as melanoma or lung cancer, is not well determined. A proposed mechanism of resistance in LMS of the uterus is the phosphatase and tensin homolog loss, which decreases the levels of genes encoding neoantigens.[81] Alternative mechanism of resistance is the loss of major histocompatibility complex I expression, which leads to immune escape.[82,83] Other proposed mechanisms are DNA methylation, epidermal growth factor receptor mutations, MYC overexpression, mutations in the phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway, activation of β-catenin, JAK1 or JAK2 mutations and PDJ amplification, depending on the form of resistance (primary, adaptive immune, acquired).[84] Based on cells' allocation, increased neutrophil-to-lymphocyte ratio and rates of TILs and TAMs

were evaluated in different studies, such as in the SARC028 trial, as additional mechanisms of resistance.[85] Finally, TLS presence seems to be a promising predictive and prognostic biomarker in STS treated with pembrolizumab.[44]

Based on the data reported, STS and BS are not responsive to ICI monotherapy. On the contrary, combinations of immunotherapy with cytotoxic chemotherapy and TKIs show promising results for several sarcoma histotypes. Additionally, from the recently published trials, ASPS has been proved to be responsive to ICI and TKI combination.[15,30,39-41,57,78] Even though it is an ultra-rare sarcoma with limited cases treated with these combinations, its therapeutic strategy should include immunotherapy. It is worth mentioning that the Food and Drug Administration recently approved atezolizumab for adult and pediatric patients aged 2 years and older with ASPS.[30,86]

Other forms of immunotherapy, which are under research in sarcomas, are interleukin, cytokines, dendritic cell vaccines, anti-CC chemokine receptor type 4, anti-transforming growth factor-β, anti-T-cell immunoglobulin and mucin domain 3 and pexa-vec.[87,88] Patients with BS and STS have a low rate of response to dendritic cell immunotherapy.[89,90] New clinical trials with well-selected populations and limited histologic and genetic heterogeneity will overcome the barriers that until now have not allowed immunotherapy to be part of the therapeutic strategy of sarcomas.

Conclusions

Although monotherapy of immunotherapeutic agents did not show encouraging results in sarcomas, combinations of them with chemotherapy or TKIs showed promising responses in different histotypes of sarcomas. As an exception, atezolizumab has been approved for patients with ASPS. Future studies will determine whether combinations of immunotherapy with other agents will be effective in specific sarcoma histotypes.

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