Maximizing Immunotherapy in Sarcoma Using Histology, Biomarkers and Novel Approaches

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Sarcomas are rare tumours with variable treatment responsiveness. Immunotherapy represents a promising approach but has yet to be fully realized. Clinical trials have shown modest response rates to checkpoint blockade (overall response rate: 5–18%) but with wide variability across histologic subtypes (up to 70% in small series). Biomarkers may help further predict response, but traditional markers are rarely expressed and less useful within sarcoma. An overarching immune classification combining several biomarkers offers better predictive validity. Exciting novel approaches include leveraging cancer testis antigens for adoptive T-cell therapies, identifying synergistic combination regimens and targeting epigenetic mechanisms to improve immune responsiveness.

Keywords

Sarcoma, immunotherapy, adoptive immunotherapy, immune checkpoint inhibitors, tumour biomarkers, tumour microenvironment

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Sarcomas are a diverse group of rare muscle, bone and connective tissue tumours. Within soft-tissue sarcoma alone, there are over 70 subtypes, which demonstrate substantial heterogeneity in biology, genetics and clinical behaviour.[1] Further complicating their management is the range of treatment responsiveness, with some subtypes wholly refractory to traditional chemotherapy, while others are quite responsive to ifosfamide or anthracycline-based regimens. Yet, even 'chemosensitive' subtypes generally demonstrate overall response rates (ORRs) of <50%.[2] Targeted therapies have emerged for several diseases, notably imatinib for gastrointestinal stromal tumours (GIST),[3] mechanistic target of rapamycin inhibitors for perivascular epithelioid cell tumours,[4,5] and anaplastic lymphoma kinase inhibitors for inflammatory myofibroblastic tumours.[6] Widespread deployment of targeted agents remains limited by the lack of driver mutations in many subtypes and incomplete biological understanding of others. The range of disease behaviour and treatment refractoriness creates an urgent need for new therapy options.

Immunotherapy has revolutionized the treatment of many malignancies, including melanoma and non-small-cell lung cancer (NSCLC), and holds great promise for sarcoma.[7,8] Immunotherapy is predominantly employed through checkpoint blockade using monoclonal antibodies against programmed death 1 (PD1), programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), in addition to oncolytic viruses and chimeric T-cell therapies. However, immunotherapy has yet to show the dramatic responses in sarcoma achieved in other cancers. The aforementioned heterogeneity leads to varied treatment responses; thus, sarcoma treatment providers are still searching for how to best incorporate immunotherapy into practice. This article outlines the evidence for the effectiveness of various immunotherapy agents as determined by histological subtype and biomarker expression, as well as newer approaches to maximize the deployment of immunotherapy in the care of patients with sarcoma.

Immunotherapy response by histology

The landmark SARCO28 trial (SARC028: A phase II study of the anti-PD1 antibody pembrolizumab [MK-3475] in patients with advanced sarcomas; ClinicalTrials.gov identifier: NCT02301039), a multicentre phase II study of pembrolizumab in advanced soft-tissue and bone sarcomas, was the first to suggest a differential response to checkpoint blockade by histological subtype.[9] While 18% of the 40 patients with soft-tissue sarcoma demonstrated objective responses, substantial differences were seen across subtypes: 40% of patients with undifferentiated pleomorphic sarcoma (UPS) had objective responses, compared with 20% in dedifferentiated liposarcoma (ddLPS), 10% in synovial sarcoma and 0% in leiomyosarcoma (LMS). Subsequent expansion cohorts reinforced these patterns, with a 23% ORR among patients with UPS (9/40) but only 10% in the ddLPS cohort (4/39).[10]

The Alliance A091401 trial (Randomized phase II study of nivolumab with or without ipilimumab in patients with metastatic or unresectable sarcoma; ClinicalTrials.gov identifier: NCT02500797) demonstrated this nuance even more strikingly.[11] Patients with advanced sarcoma were assigned to single-agent nivolumab or combination nivolumab and ipilimumab for four cycles, followed by

Table 1: Selected results of immunotherapy response by histological subtype

	ORR range from selected studies, %	Number of studies	Pooled estimate of ORR*, %	Agents tested
Undifferentiated pleomorphic sarcoma	23.0–40.0	3	15.7	Pembrolizumab, nivolumab +/- ipilimumab
Dedifferentiated liposarcoma	10.0–20.0	3	7.3	Pembrolizumab, nivolumab +/- ipilimumab
Alveolar soft-part sarcoma	37.2–50.0	4	48.8	Atezolizumab, pembrolizumab +/- axitinib, geptanolimab
Angiosarcoma	25.0–71.0	4	–	Nivolumab + ipilimumab, pembrolizumab, TVEC, others
Osteosarcoma	0.0–20.9	3	–	Pembrolizumab + cyclophosphamide, nivolumab, camrelizumab + apatinib
Leiomyosarcoma	0.0–10.3	2	–	Pembrolizumab, nivolumab +/- ipilimumab

**Previously published pooled analysis of published clinical trials, totalling 384 patients across histological subtypes treated with PD1/PD-L1 inhibitors with or without other agents.[26]*
ORR = overall response rate; PD1 = programmed death 1; PD-L1 = programmed death-ligand 1; TVEC = talimogene laherparepvec.

2 years of nivolumab. Although the results were modest when analysed in aggregate (ORR 5% for nivolumab alone, 16% for nivolumab/ipilimumab), analysis by subtype in the expansion cohorts offered a much different picture. Within UPS, 23% of patients responded to nivolumab alone and 29% to combination nivolumab and ipilimumab, rates that approximate those of first-line therapies.[12] In ddLPS, 10.0% of patients responded to nivolumab, and 17.0% responded to combination immunotherapy, outpacing the overall estimate of efficacy. Alternatively, 0 of the 18 patients with GIST and 3 of the 29 patients with LMS (10.3%) responded. These two studies highlight the need for subtype-specific evaluation of immunotherapy response, as response rates among more sensitive histologies, such as UPS and ddLPS, can be otherwise obscured.[10,11]

Subsequent research has highlighted other immune-sensitive histologies. Alveolar soft-part sarcoma (ASPS) is a rare sarcoma subtype, more common among adolescents and young adults, with consistently high response rates to checkpoint blockade.[13] A multicentre phase II study evaluating atezolizumab in 44 patients with ASPS showed an ORR of 37.2%, including one complete response (CR) and 15 partial responses (PR), which were durable for a median of 16.5 months.[14] Another 58.1% of patients had stable disease (SD). A multicentre phase II study of geptanolimab (anti-PD1 antibody) had similar findings, with an ORR of 37.8% (14/37), disease control in 86.5% (32/37) and median progression-free survival (mPFS) of 6.9 months.[15] Within the French AcSé pembrolizumab study with several cohorts of rare cancers, 50% of patients (7/14) with ASPS responded, with a 12-month PFS rate of 35.7% and 1-year overall survival of 85.7%.[16] An evaluation of pembrolizumab combined with vascular endothelial growth factor (VEGF) receptor inhibitor axitinib showed an ORR of 50% (6/12) and a mPFS of 12.4 months.[17] While point estimates and specific therapies vary slightly, these findings establish ASPS as one of the most immune-sensitive sarcoma histologies.

Angiosarcoma is a rare, aggressive, endothelial cell malignancy that also appears to be quite sensitive to immunotherapy, although the evidence base is smaller than for ASPS.[18] The dual anti-CTLA-4 and anti-PD1 blockade in rare tumours (DART) trial evaluated nivolumab in combination with ipilimumab in a multicentre phase II study across several rare cancers, including angiosarcoma.[19] Of 16 evaluable patients with angiosarcoma, four (25%) experienced an objective response. Notably, three of five patients with primary cutaneous disease responded (60% ORR). The 6-month PFS rate was 38% for the entire angiosarcoma cohort. Furthermore, a phase II study of talimogene laherparepvec in combination with pembrolizumab across several sarcoma types showed an ORR of 35%.[20] Notably, 3 of 20 patients had angiosarcoma, and two had PRs. Other findings are limited to case series but seem to confirm these results. One series from the University of North Carolina demonstrated objective responses to single-agent pembrolizumab among three of five patients with cutaneous angiosarcoma,[21] and another case series from the University of Miami described PRs to a variety of checkpoint inhibitors in five of seven patients with angiosarcoma, most of whom had cutaneous disease.[22] Clearly, the early signal is promising for checkpoint blockade with or without talimogene laherparepvec as an effective treatment strategy, particularly among those with cutaneous angiosarcoma.

By contrast, osteosarcoma has consistently been refractory to checkpoint blockade. In the PEMBROSARC study, published in 2019, pembrolizumab was evaluated with metronomic cyclophosphamide in advanced osteosarcomas.[23] Of 15 evaluable patients, only four experienced tumour shrinkage, and one had a PR (6.7%); at 6 months, 86.7% of these patients had progressed. Similar findings were seen with nivolumab when evaluated in children and young adults.[24] Among 13 patients with osteosarcoma, no objective responses were seen, nor were there any responses among 11 patients with Ewing sarcoma. The apatinib plus anti-PD1 therapy for advanced osteosarcoma (APFAO) trial evaluated apatinib plus camrelizumab (anti-PD1) for advanced osteosarcoma after progression on chemotherapy.[25] Objective responses were observed in 9 of 43 patients (20.9%), and 13 were progression free at 6 months (30.2%); thus, these patients did not meet the prespecified endpoints for benefit. Along with LMS and GIST, osteosarcoma represents an immunotherapy-resistant histology.

Table 1 shows selected results of immunotherapy responses by histological subtype.[26]

Predictive biomarkers

Numerous studies have sought reliable biomarkers that predict response to immune activation. In many cancer types, these have offered great promise.[27] PD-L1 plays a pivotal role in checkpoint blockade and is widely used as a predictive biomarker. PD-L1 expression is highly correlated with treatment response across the solid-tumour landscape, notably including NSCLC, bladder cancer, gastric cancer and others,[28] and the US Food and Drug Administration (FDA) approvals for immunotherapy agents are often contingent upon PD-L1 expression.[29] Tumour mutational burden (TMB) reflects the density of mutations within the tumour genome, and high levels of TMB are associated with response to checkpoint blockade across numerous tumour types.[30] Similarly,

tumours with defects in DNA damage repair pathways (DDR), which are characterized by elevated microsatellite instability (MSI-high), have high TMB and generally demonstrate sensitivity to immunotherapy. This was first shown in colorectal cancer but has since been seen across various tumours.[31] Given these findings, the anti-PD1 antibody pembrolizumab received disease-agnostic FDA approval for TMB-high or MSI-high/DDR-mutated tumours.[32]

Unfortunately, in sarcoma, these biomarkers have not demonstrated the same predictive ability or broad application. In the SARC028 trial, only 3 of 70 evaluable patients had a PD-L1 expression of >1%, although two of those three patients had objective responses.[9] Other studies have shown infrequent PD-L1 expression in sarcoma, as low as 0.83% according to a 2018 abstract evaluating 6,100 cases across numerous subtypes.[33] Furthermore, PD-L1 expression does not consistently predict response. In patients with ASPS treated with pembrolizumab in combination with axitinib, 52% of evaluable patients expressed PD-L1, but it did not correlate with PFS or response rate.[17] Similarly, in the atezolizumab study, 5 of 44 patients had PD-L1 expression compared with 16 responses, without clear correlation.[14] In the geptanolimab trial, 30% had a PD-L1 expression of >1%, with no difference in response between those with or without PD-L1 expression.[15]

The role of other predictive biomarkers in sarcoma is limited by a lack of robust data. In one of the only studies to evaluate TMB in sarcoma, TMB was highly predictive of the response rate to immunotherapy, with ORRs of 16% versus 75% among those with low and high TMB, respectively.[34] However, only 5% (4/84) of patients with sarcoma were TMB-high, and these findings have yet to be replicated. Similarly, MSI-high status is uncommon among sarcomas, and its predictive ability has not been evaluated.[35] DNA polymerase ε/δ 1 (*POLE/POLD1*) mutations predict immunotherapy response across cancer types, but are rarely expressed in sarcoma (2.4%), limiting generalizability.[36]

One promising approach combines several biomarkers into an overarching immune classification.[37] Given the important role of the tumour microenvironment in immune response, sarcomas were categorized based on the expression of gene signatures relating to T cells, B cells, tertiary lymphoid structures and biomarkers, including PD1, CTLA-4 and lymphocyte activation gene 3. The authors of the classification identified five distinct sarcoma immune classes from A to E, which predicted responsiveness to checkpoint blockade based on the SARC028 trial population. In class E, ORR was 50%, compared with 25%, 22% and 0% in classes D, C, and A or B, respectively. mPFS was significantly prolonged within class E compared with classes A and B (10 months versus 2 months, $p<0.05$). The immune class predicted overall survival along the same pattern. These classes track fairly closely with known histological patterns of immune responsiveness, with most LMS falling into class A or B, and most ddLPS defined as class C. Importantly, all histologies were represented in class E, suggesting that this method could discern immune-sensitive tumours better than a histology-only approach. Correspondingly, a range of PD1 expression was observed within class E. The primary barrier to implementation will be replicability. The use of common laboratory methodology in the classification (i.e. immunohistochemical analysis, flow cytometry) allows many institutions to evaluate this approach for broader utility.

Novel approaches

Perhaps the more compelling strategies in leveraging immunotherapy in sarcoma care incorporate additional targeted and cytotoxic agents to improve the efficacy of existing immune treatments, and use specific cancer antigens to target adoptive T-cell therapies. The notion of combination therapy in immuno-oncology has been present since the early trials in NSCLC, which demonstrated robust anti-tumour activity by the combination of checkpoint blockade and traditional cytotoxic chemotherapy, findings that have since been replicated in phase II and III trials.[38,39] There is a strong rationale to support the combination of chemotherapy with immunotherapy, as cytotoxic agents may increase antigen presentation by dying tumour cells, promote T-cell activation and inhibit immunosuppressive myeloid cells, among other important immune mechanisms.[30–42] Retrospective data in NSCLC have suggested that the combination of chemotherapy and immunotherapy may be more effective than immunotherapy alone, although no randomized trials have evaluated this question to date.[43] Numerous on-going sarcoma trials are currently evaluating the efficacy of combination chemoimmunotherapy, including pembrolizumab with eribulin (A phase II study of eribulin and pembrolizumab in soft tissue sarcomas; ClinicalTrials.gov identifier: NCT03899805), trabectedin with dual immune checkpoint blockade (SAINT: A phase 1/2 study of safe amounts of iplimumab, nivolumab and trabectedin as first line treatment of advanced soft tissue sarcoma [STS]; ClinicalTrials.gov identifier: NCT03138161) and an anti-CD40 monoclonal antibody with doxorubicin (A phase II trial evaluating APX005M [a CD40 agonistic monoclonal antibody] in combination with standard-of-care doxorubicin for the treatment of advanced sarcomas; ClinicalTrials.gov identifier: NCT03719430).[44–46]

There is similar enthusiasm for combining small-molecule inhibitors with immunotherapy. Preclinical data have shown synergistic effects, with inhibition of angiogenesis improving T-cell tumour infiltration and upregulating proinflammatory cytokine expression.[47–49] While early trials of the combination of VEGF inhibitors and immunotherapy in renal cell carcinoma (RCC) were limited by toxicity, the combination of axitinib and pembrolizumab has since been shown to be safe and highly effective in advanced RCC.[50,51] The combination of VEGF and checkpoint blockade has now become the standard of care for advanced RCC. On-going studies are evaluating this approach in sarcoma, including trials of lenvatinib and pembrolizumab (A pilot study of lenvatinib plus pembrolizumab in patients with advanced sarcoma; ClinicalTrials.gov identifier: NCT04784247), tivozanib and atezolizumab (Atezolizumab plus tivozanib in immunologically cold tumor types; ClinicalTrials.gov identifier: NCT05000294) and cabozantinib with dual immune checkpoint blockade (A randomized phase II trial of cabozantinib combined with PD1 and CTLA-4 inhibition in metastatic soft tissue sarcoma; ClinicalTrials.gov identifier: NCT04551430).[52–54]

One of the most cutting-edge approaches in sarcoma immunotherapy is the use of adoptive T-cell therapies. By using overexpressed cancer testis antigens (CTAs), researchers have successfully developed genetically modified T cells to specifically target malignant cells. New York-oesophageal squamous cell carcinoma-1 (NY-ESO-1) and melanoma-associated antigen-A4 (MAGE-A4) are outstanding targets for this approach, as they are frequently overexpressed in synovial sarcomas and myxoid/round-cell liposarcomas, among others, while being absent on nearly all normal tissues.[55] By harvesting autologous T cells, expanding them *ex vivo* or modifying the cells to recognize a particular CTA and then returning the cells after the patient has received a conditioning regimen, T-cell therapies have shown promising results. D'Angelo et al. evaluated autologous T cells expressing NY-ESO-1 among 12 patients with synovial sarcoma and found an ORR of 50% – including one CR – a median time to response of 6.2 weeks and a median duration of response of 30.9 weeks.[56] Nearly all patients (11/12) experienced adverse events of grade 3 or higher, relating to lymphodepletion (lymphopenia

100%, neutropenia 83%, anaemia 83%). One of the primary risks with adoptive T-cell therapies is the potential for cytokine release syndrome (CRS), in which activated T cells cause a hyperinflammatory state similar to sepsis, risking organ damage and hypoperfusion. Five of 12 patients (41.7%) experienced CRS, although no episodes were fatal and most (3/5) were low grade. Robbins et al. reported similar findings among patients with synovial sarcoma and NY-ESO-1 positivity, with an ORR of 61% (11/18).[57] Side effects were similar, with near-universal cytopenias and transient CRS, although frequency and severity were not reported. The 3- and 5-year survival rates were estimated at 38% and 14%, respectively, among patients with an incurable disease who had already progressed through standard therapies.

MAGE-A4 is another CTA in multiple solid tumours that acts to prevent cell-cycle arrest and apoptosis,[58] and researchers have seen similar success in leveraging adoptive T-cell therapies in sarcoma. In a phase I dose-escalation study, there were seven PRs (25%) and 11 SDs (39%) among 28 patients; notably, all seven PRs were in patients with synovial sarcoma.[59] Adverse effects were again largely due to the conditioning regimen, with high rates of lymphopenia, anaemia and thrombocytopenia. At the 2021 American Society of Clinical Oncology National Meeting, results were presented from the phase II trial (A phase 2 single arm open-label clinical trial of ADP-A2M4 SPEAR™ T cells in subjects with advanced synovial sarcoma or myxoid/round cell liposarcoma [SPEARHEAD-1]; ClinicalTrials.gov identifier: NCT04044768) evaluating the use of MAGE-A4 autologous T cells in patients with synovial sarcoma or myxoid/round-cell liposarcoma.[60] Of 25 evaluable patients, there were two CRs (8%), eight PRs (32%) and 11 SDs (44%). At the time of data cutoff, 90% of responders had an on-going response. Over half of the patients (59%) experienced CRS, but 95% of episodes were grade 2 or lower. Cytopenias related to the conditioning regimen were common but largely reversible.

These findings, while early, point to an important role for adoptive T-cell therapies in refractory sarcomas. The overexpression of CTAs on cancer cells but not on normal tissues is a key element of the safety and efficacy of these regimens, providing true targeted anti-tumour immunotherapy. However, there are several limitations to this approach. These modified T cells can only be used among patients who are human leukocyte antigen (HLA)-A*02:01 positive, thus limiting their generalizability.[56] Notably, this phenotype is more common among Caucasians, raising equity implications.[61] Cell processing, modification and expansion take 1–2 months, a delay that may not be feasible, depending on the clinical circumstances.[62] Durability of response remains to be seen – chimeric antigen receptor T-cell therapies in haematological malignancies have shown long-term benefit, but this has yet to be proven in sarcoma.[63]

Other strategies may potentiate existing therapies and open more treatment options to classically immune 'cold' tumours such as sarcoma, including epigenetic modification and downregulation of immune tolerance mechanisms.[64] Epigenetic changes allow cancer cells to evade immune recognition, and methylation and histone acetylation have been shown to regulate the expression of NY-ESO-1 antigens.[65] Correspondingly, the use of histone deacetylase (HDAC) inhibitors has been shown to increase NY-ESO-1 expression in tumour cells.[66] Combining HDAC inhibitors with adoptive T-cell therapies offers a clear potential synergistic benefit, enhancing the expression of cancer antigens, which can then be targeted with effective agents.

Finally, research on resistance to immunotherapy has improved our understanding of immune tolerance, a critical pathway in immune regulation with important implications for therapy. Immune tolerance is necessary for regulating intrinsic inflammatory pathways and preventing harm to normal tissues, yet cancers leverage these same pathways to evade immune detection and activation. Myeloid-derived suppressor cells (MDSCs) are one of several key actors in this process, arising in chronic inflammatory states to protect normal tissues, but are co-opted in malignancy. MDSCs directly inhibit T cells using nitric oxide, reactive oxygen species and cytokines, and seem to promote angiogenesis through the production of VEGF and related peptides, development of metastases and resistance to anti-cancer therapies.[67–70] The use of low doses of chemotherapy with gemcitabine and 5-fluorouracil has been shown to eliminate MDSCs, and targeted therapies against the tumour necrosis factor-related apoptosis-inducing ligand receptor may serve as an even more selective approach for inhibition.[71–73] Combining MDSC blockade with checkpoint inhibition may be an important mechanism for extending the viability of anti-tumour T cells and enhancing both response rates and durability of benefit.

Conclusions and future directions

While early immunotherapy trials have not led to the rapid sea change seen in melanoma and NSCLC, there is still significant promise in immunotherapy for sarcoma. Histological subtype is a simple way to narrow the breadth of sarcoma pathology to those more likely to respond to immunotherapy, particularly with ASPS, UPS and cutaneous angiosarcoma. Predictive biomarkers may allow further selection of the most sensitive tumours, as well as identify those likely to respond in traditionally immune-refractory histologies. The current standing of PD-L1, TMB and MSI in sarcoma is insufficient to guide this selection. More promising are avenues combining multiple biomarkers to determine sarcoma immune classes and leveraging the CTAs NY-ESO-1 and MAGE-A4 to develop adoptive T-cell therapies.

Ultimately, with a cancer type as varied and challenging as sarcoma, no single strategy will likely be adequate to bring immunotherapy to its full promise. Combination approaches using histology and tumour microenvironment, along with novel strategies targeting epigenetics and immune tolerance will be needed, and predictive biomarkers may ultimately differ between histological subtypes. However, caution must be taken to ensure any new stratification approach is widely applicable for patients across the USA and globally.

Finally, we need more active immunotherapy agents in sarcoma. Given the promise of checkpoint blockade, the current wave of clinical trials is evaluating mechanisms to prime sarcomas to improve immunogenicity, whether in combination with chemotherapy, tyrosine kinase inhibitors or other mechanisms. Improved therapies, more precise predictive biomarkers and continued histological stratification are needed to fully realize the promise of immunotherapy in sarcoma. ❑

1. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumours. *WHO Classification of Tumours*, Vol 3. 5th ed. Lyon: International Agency for Research on Cancer. 2020.
2. Scurr M. Histology-driven chemotherapy in soft tissue sarcomas. *Curr Treat Options Oncol*. 2011;12:32–45.
3. Dematteo RP, Ballman KV, Antonescu CR, et al. Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: A randomised, double-blind, placebo-controlled trial. *Lancet*. 2009;373:1097–104.
4. Benson C, Vitfell-Rasmussen J, Maruzzo M, et al. A retrospective study of patients with malignant PEComa receiving treatment with sirolimus or temsirolimus: The Royal Marsden Hospital experience. *Anticancer Res*. 2014;34:3663–8.
5. Wagner AJ, Ravi V, Riedel RF, et al. nab-Sirolimus for patients with malignant perivascular epithelioid cell tumors. *J Clin Oncol*. 2021;39:3660–70.
6. Mossé YP, Voss SD, Lim MS, et al. Targeting ALK with crizotinib in pediatric anaplastic large cell lymphoma and inflammatory myofibroblastic tumor: A children's oncology group study. *J Clin Oncol*. 2017;35:3215–21.
7. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378:2078–92.
8. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med*. 2017;377:1345–56.
9. Tawbi HA, Burgess M, Bolejack V, et al. Pembrolizumab in

advanced soft-tissue sarcoma and bone sarcoma (SARC028): A multicentre, two-cohort, single-arm, open-label, phase 2 trial. [published correction appears in *Lancet Oncol*. 2017;18:e711] [published correction appears in *Lancet Oncol*. 2018;19:e8]. *Lancet Oncol*. 2017;18:1493–501.
10. Burgess MA, Bolejack V, Schuetze S, et al. Clinical activity of pembrolizumab (P) in undifferentiated pleomorphic sarcoma (UPS) and dedifferentiated/pleomorphic liposarcoma (LPS): Final results of SARC028 expansion cohorts. *J Clin Oncol*. 2019;37(Suppl. 15):11015.
11. D'Angelo SP, Mahoney MR, Van Tine BA, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): Two open-label, non-comparative, randomised, phase 2 trials. *Lancet Oncol*. 2018;19:416–26.
12. D'Ambrosio L, Touati N, Blay J-Y, et al. Doxorubicin plus dacarbazine, doxorubicin plus ifosfamide, or doxorubicin alone as a first-line treatment for advanced leiomyosarcoma: A propensity score matching analysis from the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. Cancer. 2020;126:2637-47.
13. Paoluzzi L, Maki RG. Diagnosis, prognosis, and treatment of alveolar soft-part sarcoma: a review. *JAMA Oncol*. 2019;5:254-60.
14. Naqash AR, O'Sullivan Coyne GH, Moore N, et al. Phase II study of atezolizumab in advanced alveolar soft part sarcoma (ASPS). *J Clin Oncol*. 2021;39(Suppl. 15):11519.
15. Shi Y, Cai Q, Jiang Y, et al. Activity and safety of geptanolimab (GB226) for patients with unresectable, recurrent, or metastatic alveolar soft part sarcoma: A phase II, single-arm study. *Clin Cancer Res.* 2020;26:6445–52.
16. Blay J-Y, Penel N, Ray-Coquard IL, et al. High clinical activity of pembrolizumab in chordoma, alveolar soft part sarcoma (ASPS) and other rare sarcoma histotypes: The French AcSé Pembrolizumab Study from Unicancer. *J Clin Oncol*. 2021;39(Suppl. 15):11520.
17. Wilky BA, Trucco MM, Subhawong TK, et al. Axitinib plus pembrolizumab in patients with advanced sarcomas including alveolar soft-part sarcoma: A single-centre, single-arm, phase 2 trial. *Lancet Oncol*. 2019;20:837–48.
18. Young RJ, Brown NJ, Reed MW, et al. Angiosarcoma. *Lancet Oncol*. 2010;11:983-91.
19. Wagner MJ, Othus M, Patel SP, et al. Multicenter phase II trial (SWOG S1609, cohort 51) of ipilimumab and nivolumab in metastatic or unresectable angiosarcoma: A substudy of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART). *J Immunother Cancer.* 2021;9:e002990.
20. Kelly CM, Antonescu CR, Bowler T, et al. Objective response rate among patients with locally advanced or metastatic sarcoma treated with talimogene laherparepvec in combination with pembrolizumab: A phase 2 clinical trial. *JAMA Oncol*. 2020;6:402–8.
21. Stein JN, Moschos S, Grilley-Olsen J, et al. Cutaneous angiosarcoma: Clinical and molecular profile and immunotherapy in a case series. Presented at: Connective Tissue Oncology Society Annual Meeting, virtual meeting, 21 November 2019. Abstract 159.
22. Florou V, Rosenberg AE, Wieder E, et al. Angiosarcoma patients treated with immune checkpoint inhibitors: A case series of seven patients from a single institution. *J Immunother Cancer.* 2019;7:213.
23. Le Cesne A, Marec-Berard P, Blay JY, et al. Programmed cell death 1 (PD-1) targeting in patients with advanced osteosarcomas: Results from the PEMBROSARC study. *Eur J Cancer.* 2019;119:151–7.
24. Davis KL, Fox E, Merchant MS, et al. Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): A multicentre, open-label, single-arm, phase 1-2 trial. *Lancet Oncol*. 2020;21:541–50.
25. Xie L, Xu J, Sun X, et al. Apatinib plus camrelizumab (anti-PD1 therapy, SHR-1210) for advanced osteosarcoma (APFAO) progressing after chemotherapy: A single-arm, open-label, phase 2 trial. *J Immunother Cancer.* 2020;8:e000798.
26. Italiano A, Bellera C, D'Angelo S. PD1/PD-L1 targeting in advanced soft-tissue sarcomas: A pooled analysis of phase II trials. *J Hematol Oncol*. 2020;13:55.
27. Sharma P, Siddiqui BA, Anandhan S, et al. The next decade of immune checkpoint therapy. *Cancer Discov.* 2021;11:838–57.
28. Khunger M, Hernandez AV, Pasupuleti V, et al. Programmed cell death 1 (PD-1) ligand (PD-L1) expression in solid tumors as a predictive biomarker of benefit from PD-1/PD-L1 axis inhibitors: A systematic review and meta-analysis. *JCO Precis Oncol*. 2017;1:1–15.
29. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. *J Immunother Cancer.* 2019;7:278.
30. Samstein RM, Lee C-H, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet.* 2019;51:202–6.
31. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372:2509–20.
32. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357:409–13.
33. Trabucco SE, Ali SM, Sokol E, et al. Frequency of genomic biomarkers of response to immunotherapy in sarcoma. *J Clin Oncol*. 2018;36(Suppl. 15):11579.
34. Valero C, Lee M, Hoen D, et al. Response rates to anti-PD-1 immunotherapy in microsatellite-stable solid tumors with 10 or more mutations per megabase. *JAMA Oncol.* 2021;7:739–43.
35. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol.* 2017;1:1–15.
36. Wang F, Zhao Q, Wang Y-N, et al. Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. *JAMA Oncol.* 2019;5:1504–6.
37. Petitprez F, de Reyniès A, Keung EZ, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature*. 2020;577:556–60.
38. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: A randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol*. 2016;17:1497–508.
39. Lynch TJ, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: Results from a randomized, double-blind, multicenter phase II study. *J Clin Oncol*. 2012;30:2046–54.
40. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: Implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ.* 2014;21:15–25.
41. Roselli M, Cereda V, di Bari MG, et al. Effects of conventional therapeutic interventions on the number and function of regulatory T cells. *Oncoimmunology*. 2013;2:e27025.
42. Lesterhuis WJ, Punt CJA, Hato SV, et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. *J Clin Invest.* 2011;121:3100–8.
43. Wang X, Niu X, An N, et al. Comparative efficacy and safety of immunotherapy alone and in combination with chemotherapy for advanced non-small cell lung cancer. *Front Oncol.* 2021;11:611012.
44. ClinicalTrials.gov. A Phase II Study of Eribulin and Pembrolizumab in Soft Tissue Sarcomas. ClinicalTrials.gov Identifier: NCT03899805. Available at: https://clinicaltrials.gov/ct2/show/NCT03899805 (accessed 13 February 2022).
45. ClinicalTrials.gov. SAINT: Trabectedin, Ipilimumab and Nivolumab as First Line Treatment for Advanced Soft Tissue Sarcoma. ClinicalTrials.gov Identifier: NCT03138161. Available at: https://clinicaltrials.gov/ct2/show/NCT03138161 (accessed 13 February 2022).
46. ClinicalTrials.gov. APX005M and Doxorubicin in Advanced Sarcoma. ClinicalTrials.gov Identifier: NCT03719430. Available at: https://clinicaltrials.gov/ct2/show/NCT03719430 (accessed 13 February 2022).
47. Dirkx AEM, oude Egbrink MGA, Castermans K, et al. Anti-angiogenesis therapy can overcome endothelial cell anergy and promote leukocyte-endothelium interactions and infiltration in tumors. *FASEB J*. 2006;20:621–30.
48. Yasuda S, Sho M, Yamato I, et al. Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo. *Clin Exp Immunol.* 2013;172:500–6.
49. Aparicio LMA, Fernandez IP, Cassinello J. Tyrosine kinase inhibitors reprogramming immunity in renal cell carcinoma: Rethinking cancer immunotherapy. *Clin Transl Oncol*. 2017;19:1175–82.
50. Chowdhury S, McDermott DF, Voss MH, et al. A phase I/II study to assess the safety and efficacy of pazopanib (PAZ) and pembrolizumab (PEM) in patients (pts) with advanced renal cell carcinoma (aRCC). *J Clin Oncol*. 2017;35(Suppl. 15):4506.
51. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med.* 2019;380:1116–27.
52. ClinicalTrials.gov. Lenvatinib and Pembrolizumab in People with Advanced Soft Tissue Sarcoma. ClinicalTrials.gov Identifier: NCT04784247. Available at: https://clinicaltrials.gov/ct2/show/NCT04784247 (accessed 13 February 2022).
53. ClinicalTrials.gov. Atezolizumab Plus Tivozanib in Immunologically Cold Tumor Types. ClinicalTrials.gov Identifier: NCT05000294. Available at: https://clinicaltrials.gov/ct2/show/NCT05000294 (accessed 13 February 2022).
54. ClinicalTrials.gov. Cabozantinib Combined With PD-1 and CTLA-4 Inhibition in Metastatic Soft Tissue Sarcoma. ClinicalTrials.gov Identifier: NCT04551430. Available at: https://clinicaltrials.gov/ct2/show/NCT04551430 (accessed 13 February 2022).
55. Kakimoto T, Matsumine A, Kageyama S, et al. Immunohistochemical expression and clinicopathological assessment of the cancer testis antigens NY-ESO-1 and MAGE-A4 in high-grade soft-tissue sarcoma. *Oncol Lett.* 2019;17:3937–43.
56. D'Angelo SP, Melchiori L, Merchant MS, et al. Antitumor activity associated with prolonged persistence of adoptively transferred NY-ESO-1 c259T cells in synovial sarcoma. *Cancer Discov*. 2018;8:944–57.
57. Robbins PF, Kassim SH, Tran TLN, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clin Cancer Res.* 2015;21:1019–27.
58. Gjerstorff MF, Andersen MH, Ditzel HJ. Oncogenic cancer/testis antigens: prime candidates for immunotherapy. *Oncotarget.* 2015;6:15772-87.
59. Hong DS, Van Tine BA, Olszanski AJ, et al. Phase I dose escalation and expansion trial to assess the safety and efficacy of ADP-A2M4 SPEAR T cells in advanced solid tumors. *J Clin Oncol.* 2020;38(Suppl. 15):102.
60. D'Angelo SP, Van Tine BA, Attia S, et al. SPEARHEAD-1: A phase 2 trial of afamitresgene autoleucel (formerly ADP-A2M4) in patients with advanced synovial sarcoma or myxoid/round cell liposarcoma. *J Clin Oncol.* 2021;39(Suppl. 15):11504.
61. Ellis JM, Henson V, Slack R, et al. Frequencies of HLA-A2 alleles in five U.S. population groups. Predominance Of A*02011 and identification of HLA-A*0231. *Hum Immunol*. 2000;61:334-40.
62. Morotti M, Albukhari A, Alsaadi A, et al. Promises and challenges of adoptive T-cell therapies for solid tumours. *Br J Cancer.* 2021;124:1759-76.
63. Cappell KM, Sherry RM, Yang JC, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. *J Clin Oncol.* 2020;38:3805–15.
64. Murciano-Goroff YR, Warner AB, Wolchok JD. The future of cancer immunotherapy: Microenvironment-targeting combinations. *Cell Res.* 2020;30:507–19.
65. Thomas R, Al-Khadairi G, Roelands J, et al. NY-ESO-1 based immunotherapy of cancer: Current perspectives. *Front Immunol.* 2018;9:947.
66. Klar AS, Gopinadh J, Kleber S, et al. Treatment with 5-aza-2'-deoxycytidine induces expression of NY-ESO-1 and facilitates cytotoxic T lymphocyte-mediated tumor cell killing. *PLoS ONE*. 2015;10:e0139221.
67. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res.* 2017;5:3–8.
68. Tartour E, Pere H, Maillere B, et al. Angiogenesis and immunity: A bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. *Cancer Metastasis Rev.* 2011;30:83–95.
69. Toh B, Wang X, Keeble J, et al. Mesenchymal transition and dissemination of cancer cells is driven by myeloid-derived suppressor cells infiltrating the primary tumor. *PLoS Biol*. 2011;9:e1001162.
70. Feng PH, Lee KY, Chang YL, et al. CD14(+)S100A9(+) monocytic myeloid-derived suppressor cells and their clinical relevance in non-small cell lung cancer. *Am J Respir Crit Care Med*. 2012;186:1025–36.
71. Vincent J, Mignot G, Chalmin F, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res*. 2010;70:3052–61.
72. Suzuki E, Kapoor V, Jassar AS, et al. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res.* 2005;11:6713–21.
73. Condamine T, Kumar V, Ramachandran IR, et al. ER stress regulates myeloid-derived suppressor cell fate through TRAIL-R-mediated apoptosis. *J Clin Invest.* 2014;124:2626–39.