JAMA Oncology | Brief Report

Assessment of Doxorubicin and Pembrolizumab in Patients With Advanced Anthracycline-Naive Sarcoma
A Phase 1/2 Nonrandomized Clinical Trial

Seth M. Pollack, MD; Mary W. Redman, PhD; Kelsey K. Baker, MS; Michael J. Wagner, MD; Brett A. Schroeder, MD; Elizabeth T. Loggers, MD, PhD; Kathryn Trieselmann, PharmD; Vanessa C. Copeland, PharmD; Shihong Zhang, PhD; Graeme Black, BS; Sabrina McDonnell, MS; Jeffrey Gregory, BS; Rylee Johnson, BS; Roxanne Moore, BS; Robin L. Jones, MD; Lee D. Cranmer, MD, PhD

IMPORTANCE Anthracycline-based therapy is standard first-line treatment for most patients with advanced and metastatic sarcomas. Although multiple trials have attempted to show improved outcomes in patients with soft-tissue sarcoma over doxorubicin monotherapy, each has fallen short of demonstrating improved outcomes.

OBJECTIVE To evaluate the safety and efficacy of doxorubicin in combination with pembrolizumab in patients with advanced, anthracycline-naive sarcomas.

DESIGN, SETTING, AND PARTICIPANTS This nonrandomized clinical trial used a 2-stage phase 2 design and was performed at a single, academic sarcoma specialty center. Patients were adults with good performance status and end-organ function. Patients with all sarcoma subtypes were allowed to enroll with the exception of osteosarcoma, Ewing sarcoma, and alveolar and embryonal rhabdomyosarcoma.

INTERVENTIONS Two dose levels of doxorubicin (45 and 75 mg/m^2) were tested for safety in combination with pembrolizumab.

MAIN OUTCOMES AND MEASURES Objective response rate (ORR) was the primary end point. Overall survival (OS) and progression-free survival (PFS) were secondary end points. Correlative studies included immunohistochemistry, gene expression, and serum cytokines.

RESULTS A total of 37 patients (22 men; 15 women) were treated in the combined phase 1/2 trial. The median (range) patient age was 58.4 (25-80) years. The most common histologic subtype was leiomyosarcoma (11 patients). Doxorubicin plus pembrolizumab was well tolerated without significant unexpected toxic effects. The ORR was 13% for phase 2 patients and 19% overall. Median PFS was 8.1 (95% CI, 7.6-10.8) months. Median OS was 27.6 (95% CI, 18.7-not reached) months at the time of this analysis. Two of 3 patients with undifferentiated pleomorphic sarcoma and 2 of 4 patients with dedifferentiated liposarcoma had durable partial responses. Tumor-infiltrating lymphocytes were present in 21% of evaluable tumors and associated with inferior PFS (log-rank P = .03). No dose-limiting toxic effects were observed.

CONCLUSIONS AND RELEVANCE In this nonrandomized clinical trial, doxorubicin plus pembrolizumab was well tolerated. Although the primary end point for ORR was not reached, the PFS and OS observed compared favorably with prior published studies. Further studies are warranted, especially those focusing on undifferentiated pleomorphic sarcoma and dedifferentiated liposarcoma.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT02888665

JAMA Oncol. 2020;6(11):1778-1782. doi:10.1001/jamaoncol.2020.3689
Published online September 10, 2020.

Supplemental content

Author Affiliations: Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington (Pollack, Redman, Baker, Wagner, Schroeder, Loggers, Trieselmann, Copeland, Zhang, Black, McDonnell, Gregory, Johnson, Moore, Cranmer); Division of Oncology, University of Washington, Seattle, Washington (Pollack, Redman, Wagner, Loggers, Trieselmann, Copeland, McDonnell, Gregory, Johnson, Moore, Cranmer); Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington (Redman); Department of Internal Medicine, Virginia Mason Medical Center, Seattle, Washington (Schroeder); Royal Marsden Hospital and Institute of Cancer Research, London, England (Jones).

Corresponding Author: Seth M. Pollack, MD, Fred Hutchinson Cancer Research Center, Clinical Research Division, 1100 Fairview Ave, D3-100, Seattle, WA 98109 (spollack@fredhutch.org).

Patients with advanced soft-tissue sarcoma (STS) generally receive first-line therapy with doxorubicin alone or with ifosfamide.[1] Median overall survival (OS) for doxorubicin alone ranges from 12.8 to 20.4 months, with median progression-free survival (PFS) of 4.1 to 6.8 months and objective response rates (ORRs) of 12% to 20%.[1-5] Multiple trials combining doxorubicin with investigational agents have failed to show improved outcomes.[1-6]

Programmed cell death ligand 1 (PD-L1) is variably expressed by STS, especially undifferentiated pleomorphic sarcomas (UPSs).[7,8] Responses to pembrolizumab monotherapy have been seen in STS, particularly UPS,[9] and some, though not all,[10] combination therapies may be associated with improved programmed cell death 1 (PD-1) blockade.[11-13] Chemotherapy may synergize with immunotherapy by depleting suppressive immune cells, releasing damage-associated molecular patterns and increasing tumor antigen presentation through tumor toxicity.[14] We conducted a phase 1/2 trial to assess the tolerability and outcomes of treatment with pembrolizumab in combination with doxorubicin.

Key Points

Question Is the combination of doxorubicin and pembrolizumab an effective and feasible regimen for patients with advanced sarcoma?

Findings In this nonrandomized phase 1/2 clinical trial of 37 patients with advanced sarcoma, the combination of doxorubicin and pembrolizumab was well tolerated. The objective response rate was 13% for phase 2 patients and 19% overall, with median progression-free survival of 8.1 months and median overall survival of 27.6 months.

Meaning Doxorubicin in combination with pembrolizumab is a promising combination worthy of further study, especially in certain sarcoma subtypes, including undifferentiated pleomorphic sarcoma and dedifferentiated liposarcoma.

Methods

Patients, Treatment Schedules, and Supportive Care

All research herein was reviewed and approved by the Fred Hutchinson Cancer Research Center Institutional Review Board. All patients provided written informed consent in accordance with the Helsinki Declaration. The trial protocol can be found in Supplement 1. Race and ethnicity data were self-reported and collected for institutional reporting requirements.

Pathology was confirmed at the University of Washington. Adult patients with sarcoma with Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ function were eligible. Patients with osteosarcoma, Ewing sarcoma, and alveolar and embryonal rhabdomyosarcoma were ineligible because well-established alternative regimens exist (eFigure 1 in Supplement 2).

Patients' initial cycle was pembrolizumab (200 mg administered intravenously) alone. Cycles were 21 days. Starting with cycle 2, doxorubicin was given prior to pembrolizumab, same day, every 3 weeks, for up to 6 cycles. After cycle 7, pembrolizumab treatment continued for up to 2 years (see eFigure 2A in Supplement 2). Growth factors were not permitted until cycle 3 during phase 1. Imaging, performed every 12 weeks, was assessed by Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 with the option of confirming progressive disease (PD) in clinically well patients.

Trial Design, Statistical Basis, and End Points

Two doses of doxorubicin (45 and 75 mg/m^2) were evaluated with pembrolizumab using a 3 + 3 design. A dose-limiting toxic effect was defined as any possibly related, unexpected, grade 3 or greater serious adverse events, or any possibly related adverse event requiring discontinuation of either drug during the first 6 weeks of combination treatment (eFigure 2B in Supplement 2).

The primary end point of the phase 2 portion of the study was the ORR as assessed by RECIST 1.1.[15] The 2-stage study was designed to rule out ORR of 15% or less with 85% power if the true ORR was 35%, using a 1-sided 5% level test. If 2 or more responses were observed in the 20-patient first stage, an additional 15 patients would accrue. If 10 or more responses of 35 patients (29%) were observed, this would rule out a 15% ORR. Although the primary end points were evaluated for the phase 1 and 2 cohorts separately, data were combined in other analyses. Data were analyzed as of September 2019.

The OS was calculated as the duration from the start of treatment to death due to any cause, and PFS as the duration from start to progression or death. Outcomes were censored at the date of last contact for living patients (OS) or living and progression free (PFS). For these secondary end points, 2-sided *P* values less than .05 were considered significant. All analyses were performed using SAS, version 9.4 (SAS Institute); Excel, version 16.33 (Microsoft Corp); and Prism, version 8.4 (GraphPad Software) (see eFigure 3 in Supplement 2 for detailed methods regarding correlative analyses).

Results

Patient Demographic Characteristics

In the combined phase 1/2 trial, 37 patients (22 men; 15 women) were treated, including 6 phase 1 patients—3 patients at each dose. Both phase 2 stages enrolled, but accrual was closed at 31 of 35 planned patients because of an insufficient number of second-stage partial responses (PRs), indicating that the study would not achieve the primary end point. The most common histology was leiomyosarcoma, present in 11 patients (30%), 3 being uterine leiomyosarcomas. Demographic characteristics, including histology, are summarized in **Table 1.**

Safety

No dose-limiting toxic effects were observed. The phase 2 dose was 75 mg/m^2. In both phase 1 and 2 cohorts, the most

Table 1. Patient Demographic Characteristics

Characteristic	No. (%) (N = 37)
Age at day 1, median (range), y	58.4 (25.8-80.4)
Sex	
Female	15 (41)
Male	22 (59)
Race	
American Indian or Alaska Native	2 (6)
Asian	2 (6)
Black or African American	1 (3)
White/other	32 (85)
No. of prior lines of systemic treatment	
0	28 (76)
1	7 (19)
2	2 (5)
Doxorubicin dose, mg/m^2	
45	3 (8)
75	34 (92)
Best response	
Not evaluable	1 (3)
Partial response	7 (19)
Progressive disease	7 (19)
Stable disease	22 (59)
Disease	
Alveolar soft-part sarcoma	1 (3)
Angiosarcoma	1 (3)
Clear cell chondrosarcoma	1 (3)
Conventional chondrosarcoma	3 (8)
Dedifferentiated liposarcoma	4 (11)
Endometrial stromal sarcoma	2 (5)
Epithelioid hemangioendothelioma	2 (5)
Epithelioid sarcoma	1 (3)
Extraskeletal myxoid chondrosarcoma	1 (3)
Hemangiopericytoma	1 (3)
Leiomyosarcoma	11 (30)
Myxofibrosarcoma	1 (3)
Pleomorphic liposarcoma	1 (3)
Pleomorphic rhabdomyosarcoma	1 (3)
Solitary fibrous tumor	2 (5)
Spindle cell sarcoma	1 (3)
Undifferentiated pleomorphic sarcoma	3 (8)

common toxic effects were nausea (n = 32) and fatigue (n = 21) (see **Table 2**; eTable 1 in Supplement 2). No grade 5 toxic effects were seen; the only attributable grade 4 toxic effects were neutropenia (n = 6), leukopenia (n = 1), and febrile neutropenia (1), all of which resolved. Two patients had grade 3 reductions in ejection fraction attributable to doxorubicin. Notable pembrolizumab-related toxic effects included grade 3 adrenal insufficiency (n = 1) and hypothyroidism (n = 7).

Tumor Response

In the combined phase 1/2 trial, confirmed PRs were seen in 7 of 37 patients (19%), 4 in the phase 2 cohort (13%) and 3 in the 75 mg/m^2 phase 1 cohort (**Figure**, A). Two patients had unconfirmed PRs, and 11 patients had stable disease with tumor regression as their best response (Figure, B). One patient came off study for increasing symptoms prior to follow-up imaging. Two of 3 patients with UPS, and 2 of 4 patients with dedifferentiated liposarcoma had durable PRs (eFigure 4 in Supplement 2). Three patients with chondrosarcoma had tumor regression, including 1 conventional chondrosarcoma with a 26% decrease in size.

Survival Outcomes

Median PFS was 8.1 (95% CI, 7.6-10.8) months, with 29 of 37 patients (78.4%) having had an event and 4 patients with continuing stable disease or PR at the time of this analysis. The PFS rates at 12 and 24 weeks were 81% (95% CI, 64%-90%) and 73% (95% CI, 56%-84%), respectively. At 12 months, the PFS was 27% (95% CI, 14%-42%). Median OS was 27.6 (95% CI, 18.7-not reached) months at the time of this analysis.

Correlative Studies

Immunohistochemistry was evaluable for 29 patients; 66% had PD-L1 expression scores of 0, reflecting a low level of PD-L1 expression (eTable 2 in Supplement 2). Expression of PD-L1 was not associated with PFS or OS. Tumor-infiltrating lymphocytes were present in 21% of evaluable tumors and associated with inferior PFS (log-rank P = .03) (eFigure 5 in Supplement 2). This was confirmed in a multivariate Cox regression analysis that adjusted for age, sex, and number of prior therapies (P = .04; eTable 3 in Supplement 2). Nanostring data were available for 24 patients (eTable 4 in Supplement 2). No gene was significantly associated with PFS after correction for multiple comparisons.

Serum cytokine levels were assessed before treatment and during cycles 1 and 2. Granulocyte macrophage-colony-stimulating factor levels increased each cycle, and IL-15 levels dropped following doxorubicin treatment. Circulating IL-2R, IP10, and CD30 levels rose sharply after cycle 1, while levels of IL-8 dropped.

Discussion

This nonrandomized phase 1/2 trial demonstrated that doxorubicin plus pembrolizumab can be given safely and may be associated with clinical benefit for patients with advanced sarcoma. While the combined ORR observed here is similar to prior published series, the PFS and OS seen here are encouraging.[2-4,6] In correlative studies, we found that tumor-infiltrating lymphocytes were associated with inferior PFS. This may reflect more aggressive tumor biology rather than as association with the PD-1 inhibitor.

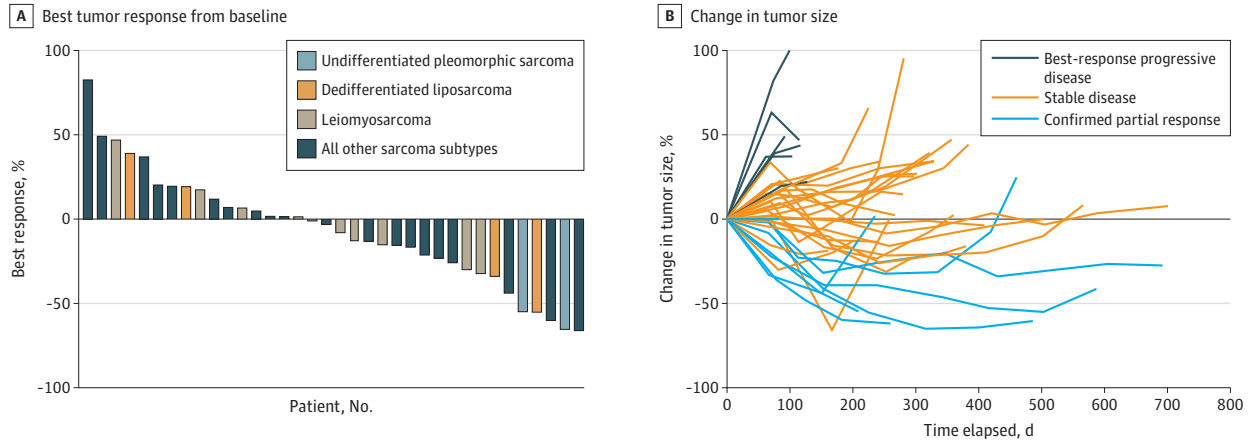
Limitations

Similar to other sarcoma trials, histologic subtypes likely influenced these results. Our trial, like others, demonstrated a higher response rate and clinical benefit of pembrolizumab in UPS compared with other sarcoma subtypes,[11] despite their generally worse prognosis.[5]

Table 2. Adverse Events

Adverse event	No. Grade 1	2	3	4	Total No. of events
Adverse events with at least 2 grade 3 or 4 events					
Ejection fraction decreased	0	0	2	0	2
Lymphocyte count decreased	0	0	2	0	2
Febrile neutropenia	0	1	1	1	3
White blood cell count decreased	0	0	2	1	3
Anemia	2	1	2	0	5
Neutrophil count decreased	0	1	2	6	9
Mucositis, oral	3	7	3	0	13
Anorexia	9	7	2	0	18
Other adverse events with at least 4 events					
Rash, maculopapular	2	2	0	0	4
Upper respiratory tract infection	2	2	0	0	4
Dyspnea	3	1	0	0	4
Headache	3	1	0	0	4
Constipation	4	1	0	0	5
Rash	4	2	0	0	6
Hypomagnesemia	6	0	0	0	6
Weight loss	4	1	1	0	6
Fever	6	1	0	0	7
Hypothyroidism	2	4	1	0	7
Dry eye	7	1	0	0	8
Diarrhea	5	2	1	0	8
Pruritus	8	1	0	0	9
Dysgeusia	7	3	0	0	10
Vomiting	5	6	0	0	11
Dry mouth	10	1	0	0	11
Alopecia	4	10	0	0	14
Fatigue	11	10	0	0	21
Nausea	15	16	1	0	32

Figure. Patient Responses



A, Waterfall plot demonstrating the percentage change in tumor size from baseline constituting the best response for each patient. B, Spider plots showing responses for all patients.

Durable PRs were also seen in 2 of 4 patients with dedifferentiated liposarcoma. Chondrosarcomas are generally resistant to doxorubicin; inclusion of these patients in the study likely lowered the ORR. Still, 2 patients with chondrosarcoma had durable disease regression, suggesting that doxorubicin/pembrolizumab may benefit these patients. For patients with these selected subtypes, follow-up studies are warranted.

ARTICLE INFORMATION

Accepted for Publication: June 9, 2020.

Published Online: September 10, 2020. doi:10.1001/jamaoncol.2020.3689

Author Contributions: Dr Pollack had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Pollack, Trieselmann, Jones, Cranmer.
Acquisition, analysis, or interpretation of data: Pollack, Redman, Baker, Wagner, Schroeder, Loggers, Copeland, Zhang, Black, McDonnell, Gregory, Johnson, Moore, Jones, Cranmer.
Drafting of the manuscript: Pollack, Redman, McDonnell, Gregory, Moore, Jones.
Critical revision of the manuscript for important intellectual content: Pollack, Baker, Wagner, Schroeder, Loggers, Trieselmann, Copeland, Zhang, Black, McDonnell, Gregory, Johnson, Moore, Jones, Cranmer.
Statistical analysis: Pollack, Redman, Baker, Schroeder, Jones, Cranmer.
Obtained funding: Pollack, Moore, Jones.
Administrative, technical, or material support: Loggers, Copeland, Zhang, Black, McDonnell, Gregory, Johnson, Moore, Jones, Cranmer.
Study supervision: Pollack, McDonnell, Gregory, Johnson, Moore, Jones.

Conflict of Interest Disclosures: Dr Pollack reported research funding from Merck during the conduct of the study; research funding from EMD Serono, Incyte, Presage, Janssen, OncoSec, and Juno and consulting, honoraria, and advisory activity with GlaxoSmithKline, Eli Lilly and Company, Seattle Genetics, Bayer, Tempus, Daiichi Sankyo, and Blueprint Medicine outside the submitted work. Dr Redman reported grants and other from Merck outside the submitted work. Dr Wagner reported research funding from Athenex, Deciphera, Incyte, Tempus, Adaptimmune, and GlaxoSmithKline and consulting, honoraria, and advisory activity with Tempus, Deciphera, and Adaptimmune outside the submitted work. Mr Black reported grants from Merck during the conduct of the study. Mr Gregory and Mss McDonnell, Johnson, and Moore reported grants and nonfinancial support from Merck & Co during the conduct of the study. Dr Jones reported grants from Merck during the conduct of the study; research support from Merck Sharp & Dohme and GlaxoSmithKline and consultation fees from Adaptimmune, Athenex, Blueprint Medicine, Clinigen, Eisai, Epizyme, Daiichi Sankyo, Deciphera, Immune Design, Eli Lilly and Company, Merck, Pharma Mar, and UpToDate outside the submitted work; in addition, Dr Jones had a patent to biomarker, issued. Dr Cranmer reported grants from Merck during the conduct of the study; research funding from Eli Lilly and Company, AADi, Blueprint Medicine, Iterion, Gradalis, Philogen, Advenchen Laboratories, and CBA Pharma; research funding to his institution from Eli Lilly and Company; and honoraria or advisory board membership for Blueprint Medicines, Daiichi Sankyo, and Regeneron outside the submitted work. No other disclosures were reported.

Funding/Support: Merck provided both funding and pembrolizumab to support this trial. Dr Pollack was also supported by the National Cancer Institute (R01CA244872), the Department of Defense (CA180380), and a grant from the V Foundation.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Meeting Presentation: This work was presented, in part, at the 2019 Annual Meeting of the American Society of Clinical Oncology; May 31-June 4, 2019; Chicago, Illinois.

Additional Contributions: The authors would like to thank Steven Townson, PhD, and Michael Shahnazari, PhD, of Merck for their many significant contributions to this study and the manuscript. They received compensation as employees of Merck.

REFERENCES

1. Judson I, Verweij J, Gelderblom H, et al; European Organisation and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. *Lancet Oncol*. 2014;15(4):415-423. doi:10.1016/S1470-2045(14)70063-4

2. Ryan CW, Merimsky O, Agulnik M, et al. PICASSO III: a phase III, placebo-controlled study of doxorubicin with or without palifosfamide in patients with metastatic soft tissue sarcoma. *J Clin Oncol*. 2016;34(32):3898-3905. doi:10.1200/JCO.2016.67.6684

3. Tap WD, Papai Z, Van Tine BA, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2017;18(8):1089-1103. doi:10.1016/S1470-2045(17)30381-9

4. Tap WD, Jones RL, Van Tine BA, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: an open-label phase 1b and randomised phase 2 trial. *Lancet*. 2016;388(10043):488-497. doi:10.1016/S0140-6736(16)30587-6

5. Seddon B, Strauss SJ, Whelan J, et al. Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. *Lancet Oncol*. 2017;18(10):1397-1410. doi:10.1016/S1470-2045(17)30622-8

6. Tap WD, Wagner AJ, Schöffski P, et al; ANNOUNCE Investigators. Effect of doxorubicin plus olaratumab vs doxorubicin plus placebo on survival in patients with advanced soft tissue sarcomas: the ANNOUNCE randomized clinical trial. *JAMA*. 2020;323(13):1266-1276. doi:10.1001/jama.2020.1707

7. D'Angelo SP, Shoushtari AN, Agaram NP, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. *Hum Pathol*. 2015;46(3):357-365. doi:10.1016/j.humpath.2014.11.001

8. Pollack SM, He Q, Yearley JH, et al. T-cell infiltration and clonality correlate with programmed cell death protein 1 and programmed death-ligand 1 expression in patients with soft tissue sarcomas. *Cancer*. 2017;123(17):3291-3304. doi:10.1002/cncr.30726

9. Tawbi HA-H, Burgess MA, Crowley J, et al. Safety and efficacy of PD-1 blockade using pembrolizumab in patients with advanced soft tissue (STS) and bone sarcomas (BS): results of SARC028—a multicenter phase II study [abstract 11006]. *J Clin Oncol*. 2016;34(15)(suppl). doi:10.1200/JCO.2016.34.15_suppl.11006

10. Toulmonde M, Penel N, Adam J, et al. Use of PD-1 targeting, macrophage infiltration, and IDO pathway activation in sarcomas: a phase 2 clinical trial. *JAMA Oncol*. 2018;4(1):93-97. doi:10.1001/jamaoncol.2017.1617

11. 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. *Lancet Oncol*. 2017;18(11):1493-1501. doi:10.1016/S1470-2045(17)30624-1

12. 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(6):837-848. doi:10.1016/S1470-2045(19)30153-6

13. 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. doi:10.1001/jamaoncol.2019.6152

14. Pollack SM, Ingham M, Spraker MB, Schwartz GK. Emerging targeted and immune-based therapies in sarcoma. *J Clin Oncol*. 2018;36(2):125-135. doi:10.1200/JCO.2017.75.1610

15. Eisenhauer EA, Therasse P, Bogaerts J, et al. New Response Evaluation Criteria in Solid Tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247. doi:10.1016/j.ejca.2008.10.026