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Atezolizumab for advanced alveolar soft part sarcoma

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ORIGINAL ARTICLE

Atezolizumab for Advanced Alveolar Soft Part Sarcoma

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ABSTRACT

BACKGROUND

Alveolar soft part sarcoma (ASPS) is a rare soft-tissue sarcoma with a poor prognosis and no established therapy. Recently, encouraging responses to immune checkpoint inhibitors have been reported.

METHODS

We conducted an investigator-initiated, multicenter, single-group, phase 2 study of the anti–programmed death ligand 1 (PD-L1) agent atezolizumab in adult and pediatric patients with advanced ASPS. Atezolizumab was administered intravenously at a dose of 1200 mg (in patients ≥18 years of age) or 15 mg per kilogram of body weight with a 1200-mg cap (in patients <18 years of age) once every 21 days. Study end points included objective response, duration of response, and progression-free survival according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, as well as pharmacodynamic biomarkers of multistep drug action.

RESULTS

A total of 52 patients were evaluated. An objective response was observed in 19 of 52 patients (37%), with 1 complete response and 18 partial responses. The median time to response was 3.6 months (range, 2.1 to 19.1), the median duration of response was 24.7 months (range, 4.1 to 55.8), and the median progression-free survival was 20.8 months. Seven patients took a treatment break after 2 years of treatment, and their responses were maintained through the data-cutoff date. No treatment-related grade 4 or 5 adverse events were recorded. Responses were noted despite variable baseline expression of programmed death 1 and PD-L1.

CONCLUSIONS

Atezolizumab was effective at inducing sustained responses in approximately one third of patients with advanced ASPS. (Funded by the National Cancer Institute and others; ClinicalTrials.gov number, NCT03141684.)

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Alveolar soft part sarcoma (ASPS) is a rare cancer that accounts for less than 1% of all soft-tissue sarcomas. With a global incidence of less than 1 per 1,000,000 persons, ASPS has been classified by the Connective Tissue Oncology Society as an ultrarare sarcoma.[1] The disease typically occurs in adolescents and young adults. ASPS generally has an indolent course but has a poor prognosis and a high tendency for early metastatic spread, which is associated with 5-year overall survival ranging from 20 to 46%.[2] The defining molecular event in ASPS is a chromosomal translocation, der(17)t(X;17)(p11;q25), involving *ASPL* (also known as *ASPSCR1*) and *TFE3*.[3] Two distinct ASPL–TFE3 fusion proteins have been identified that reportedly differ in transcriptional activity.[4] However, to date, no clinical significance with respect to prognosis has been described for type 1 fusions as compared with type 2 fusions.[5]

When this study began, there was no established therapy for ASPS. The disease is largely resistant to traditional chemotherapies. Initial management through surgical resection, systemic treatment, or both is rarely curative.[2] The tyrosine kinase inhibitor pazopanib is approved by the Food and Drug Administration (FDA) for soft-tissue sarcoma in general and is a treatment option for patients with ASPS. In December 2022, on the basis of independently reviewed results from the study reported herein, the FDA approved atezolizumab, an immune checkpoint inhibitor targeting programmed death ligand 1 (PD-L1), for the treatment of unresectable or metastatic ASPS in adult patients and pediatric patients 2 years of age or older.[6]

Here, we report the clinical and pharmacodynamic results of a single-group, phase 2 clinical study of an immunotherapy agent, atezolizumab, for the treatment of advanced ASPS. Encouraging clinical responses in persons with ASPS to other immune checkpoint inhibitors that disrupt PD-L1 binding to its programmed death 1 (PD-1) receptor, with or without a tyrosine kinase inhibitor, have been reported.[7-20] However, most sample sizes were small, and the molecular mechanisms behind the responses were unclear. Tumor mutational burden and microsatellite instability, which are associated with the presence of tumor neoantigens and a high likelihood of response to immune checkpoint inhibition in many histologic types of cancer,[21] are low in ASPS.[8,11,22,23] In addition, some ASPS tumors that are responsive to immune checkpoint inhibitors reportedly lack the expression of PD-L1 or PD-1 proteins that are associated with response to immune checkpoint blockade.[9-11,23,24] Therefore, we conducted the present study to evaluate the efficacy of atezolizumab treatment for ASPS and to investigate the hypothesis that the mechanism of activity of immune checkpoint inhibitors in this disease involves induced expression of missing immune checkpoint components.

METHODS

STUDY DESIGN AND OVERSIGHT

Eligible patients were 2 years of age or older with histologically or cytologically confirmed ASPS (including newly diagnosed, unresectable or metastatic, and measurable disease with clinical evidence of disease progression) who had received no previous anti–PD-1 or anti–PD-L1 therapy. Atezolizumab was administered intravenously at a dose of 1200 mg (adult patients, ≥18 years of age) or 15 mg per kilogram of body weight with a 1200-mg cap (pediatric patients, <18 years of age) on the first day of each 21-day cycle. Atezolizumab doses were allowed to be held for up to 12 weeks for resolution of toxic effects; dose modifications were not permitted, given the lack of a clear atezolizumab dose–response relationship. Details regarding eligibility, dose interruptions, and treatment discontinuation are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Tumor biopsies, which were made mandatory later in the study, were performed at baseline and just before cycle 3 day 1 in patients 18 years of age or older. Circulating tumor cells were obtained from patients 14 years of age or older at baseline, on cycle 1 day 8, before the atezolizumab dose was administered at the start of each subsequent cycle, and at the time of disease progression. Patients who had received treatment for more than 2 years had the option of taking a break from treatment while retaining the chance to resume therapy at the discretion of the study investigators or on evidence of progressive disease. Patient monitoring and response assessments continued throughout the break. Patients who completed 2 years of a treatment break without resuming

atezolizumab were removed from the treatment portion of the study and were assessed every 6 months.

This investigator-initiated study was conducted at multiple sites under a National Cancer Institute (NCI)–sponsored Investigational New Drug application and was approved by the central institutional review board of the NCI. Genentech (a member of the Roche Group) collaborated in the design of the study and provided funding and atezolizumab to the NCI through a Cooperative Research and Development Agreement. The study was conducted in full accordance with Good Clinical Practice guidelines, the principles of the Declaration of Helsinki, and all applicable regulations, guidance, and local policies. Written informed consent was obtained from each adult patient and from the parent or guardian of each pediatric patient. Pediatric patients were included in discussions about the study, and their oral or written assent was obtained. The authors vouch for the accuracy and completeness of the data and for the adherence of the study to the protocol, which is available at NEJM.org.

END POINTS AND ASSESSMENTS

To determine the occurrence of objective response, duration of response, and progression-free survival, tumor response to the drug was assessed by investigator review at each study site with the use of Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.[25] Immune RECIST (iRECIST), which reflect the propensity of immune-modulating agents to elicit strong tumor responses after initial evidence of disease progression,[26] were also used as an exploratory end point. Tumor measurements for response assessment were conducted at baseline, at the end of cycle 3, every two cycles thereafter for the first year, every three cycles during the second year, and every four cycles after that. Tumors were measured with the use of computed tomography or, in select cases, magnetic resonance imaging or positron-emission tomography–computed tomography. Confirmatory scans were performed at least 4 weeks after initial documentation of objective response. Patients showing signs of clinical benefit could continue atezolizumab treatment after progression according to RECIST, version 1.1, if they met protocol-specified criteria for drug continuation. For the patients treated beyond the time of RECIST-assessed progression, all subsequent responses were assessed with the use of iRECIST.

Adverse events were reported according to NCI Common Toxicity Criteria, version 4.0, until March 31, 2018, when version 5.0 was subsequently implemented and used. All previous adverse events were mapped to version 5.0 before the final analysis.

Paired biopsy specimens (obtained before treatment and on cycle 3 day 1) were collected to evaluate the multistep mechanism of action of atezolizumab. The biopsy specimens were flash-frozen on collection, thawed under formalin fixative, and processed for analysis of sections of paraffin blocks in a manner that preserves labile phosphoproteins (see the Supplementary Appendix).[27,28] Immunofluorescence microscopy of biopsy sections that were stained with multiplexed antibody panels (PD-L1/CD8, PD-1/CD3, CD4/FOXP3, and CD8/CD3ζ pY142/TFE3) used the PD-L1 monoclonal antibody clone 73-10, which provides greater sensitivity than the VENTANA SP142 clone used in the diagnostic assay (Table S1 in the Supplementary Appendix).[29] Image-analysis algorithms identified and quantified biomarker-positive cells per square millimeter within the entire tumor area and at stroma interfaces.

PD-L1 expression in circulating tumor cells was analyzed by means of imaging flow cytometry. Type 1 and type 2 *ASPL–TFE3* fusions were identified retrospectively with the use of RNA extracted from formalin-fixed, paraffin-embedded tissue and paraformaldehyde-fixed peripheral-blood samples. (Additional details are provided in the Supplementary Appendix.)

STATISTICAL ANALYSIS

This phase 2 study used a Simon optimal two-stage design; the study was to be stopped for futility if no responses were measured among the first 9 patients. Otherwise, 15 additional patients were to be enrolled. If at least three responses were observed among the 24 total patients, the regimen was to be considered worthy of further testing in this disease. This design has 90% power to reject a null hypothesis of an incidence of response of 5% when the true incidence of response is 25% (with a one-sided type I error of 9.3%). All eligible patients who received at least one dose of study medication were included in the interim

Table 1. Demographic and Clinical Characteristics of Evaluable Patients at Baseline.*

Characteristic	Patients (N = 52)
Age — no. (%)	
2–17 yr	3 (6)
18–39 yr	37 (71)
40–70 yr	12 (23)
Sex — no. (%)	
Female	26 (50)
Male	26 (50)
Race — no. (%)†	
Black	15 (29)
Asian	5 (10)
White	27 (52)
Unknown or not reported	5 (10)
ECOG performance-status score — no. (%)‡	
0	27 (52)
1	24 (46)
2	1 (2)
ASPSL–TFE3 fusion type — no. (%)	
Type 1	30 (58)
Type 2	4 (8)
Undetermined§	18 (35)
Previous therapy — no. (%)	
None	25 (48)
Tyrosine kinase inhibitor	25 (48)
Interferon alfa	2 (4)
Other or unspecified systemic therapy	10 (19)
Surgery	34 (65)
No. of previous lines of systemic therapy — median (range)	1 (0–8)

* Percentages may not total 100 because of rounding.
† Race was reported by adult patients or by the parent or guardian of pediatric patients.
‡ Eastern Cooperative Oncology Group (ECOG) performance-status scores range from 0 to 5, with higher scores indicating greater disability.
§ The *ASPL–TFE3* fusion type could not be determined owing to the lack of a decipherable gel band after reverse-transcriptase–polymerase-chain-reaction assay (in nine patients) or the lack of sufficient biopsy or blood specimens to test (in nine patients).

and primary analyses of the incidence of response. Proceeding to the second stage of the two-stage design required approval from the study sponsor, principal investigator, and statistician. After completion of the initial two-stage design, the enrollment ceiling was increased to 53 to ensure collection of at least 10 evaluable research biopsy pairs to confirm the drug mechanism of action. This new enrollment ceiling accounted for the possibilities of unpaired biopsy specimens, biopsies of insufficient quality, and the enrollment of patients who might not contribute biopsy specimens (e.g., pediatric patients).

RESULTS

PATIENTS

From April 2017 through July 2022, a total of 53 patients were enrolled across 17 centers; 52 received treatment (Table 1). The racial diversity within the cohort reflects the racial distribution of patients with advanced ASPS, with Black patients disproportionately affected (Table S2). The median age of the adult patients at enrollment was 33 years (range, 18 to 70). Three pediatric patients were enrolled; their ages ranged from 12 to 17 years. A total of 34 patients (65%) had undergone previous resection of their primary or metastatic disease. A total of 27 patients (52%) had received at least one line of systemic therapy before enrollment; of these 27 patients, 25 (93%) had received tyrosine kinase inhibitor therapy. As an exploratory objective, the *ASPL–TFE3* fusion type of 34 patients was determined retrospectively; 30 patients (88%) expressed the type 1 fusion, and 4 patients (12%) expressed the type 2 fusion (Table S3).

EFFICACY

The regimen was considered to be worthy of further testing after eight confirmed responses were observed among the first 19 patients. Enrollment continued to allow collection of biopsy specimens to assess the mechanism of action of atezolizumab in ASPS. As of the data-cutoff date (July 31, 2022), the median time in the study was 13.2 months (range, 1.8 to 58.0) (Fig. 1). Seven patients took a treatment break after 2 years of therapy (range of break durations, 0.4 to 25.3 months [two completed and five ongoing]).

Among the 52 evaluable patients, an objective response occurred in 19 (37%; 95% binomial confidence interval [CI], 24 to 51), with 18 patients having a confirmed partial response as their best response and 1 patient having a confirmed complete response (Fig. 1). The time to a complete response was 11.8 months; after the data-cutoff date for this article, a second patient

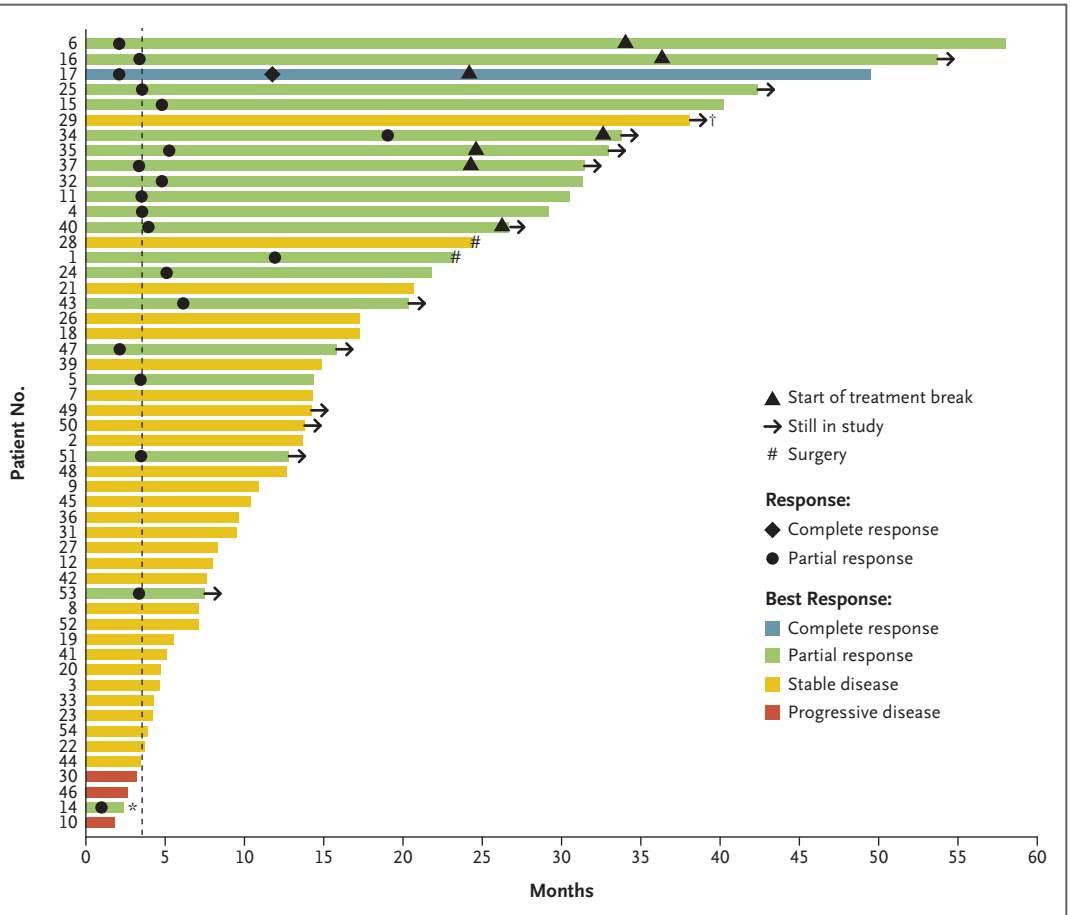


Figure 1. Patient Responses to Atezolizumab.

Time in the study (in months) as of July 31, 2022, is shown for each patient. The colors of the bars indicate the best response for each patient, and the time of the first complete or partial response is indicated by a diamond or circle, respectively. The dashed line indicates the median time to first response (3.6 months). One patient (asterisk) had an unconfirmed partial response. One patient (dagger) had a partial response according to Immune Response Evaluation Criteria in Solid Tumors (iRECIST).

had a complete response 13.7 months into treatment. Among the 19 patients with a confirmed response according to RECIST, version 1.1, the median time to response was 3.6 months (range, 2.1 to 19.1), the median duration of response was 24.7 months (range, 4.1 to 55.8), and the median best change from baseline in tumor size was −66.2% (range, −36.6 to −100) (Fig. 2). The best clinical outcomes for the other 33 patients included a partial response that was not confirmed before the patient discontinued the study (1 patient), a partial response according to iRECIST (1 patient), stable disease (28 patients), and progressive disease (3 patients). The median progression-free survival was 20.8 months (Fig. 3).

A post hoc assessment of objective response according to previous exposure to tyrosine kinase inhibitors was also performed (Fig. S1). The percentage of patients with a response was similar among patients who had previously received tyrosine kinase inhibitor treatment (36%; 95% binomial CI, 18 to 57) and among those who had not (37%; 95% binomial CI, 19 to 58).

Many patients' tumors continued to shrink over long periods of time. Two patients received treatment for approximately 12 months before their disease trajectory improved suddenly. One of these patients (Patient 29) continued treatment beyond the time of RECIST-assessed progression and was assessed subsequently by means of iRECIST, which resulted in a designation of

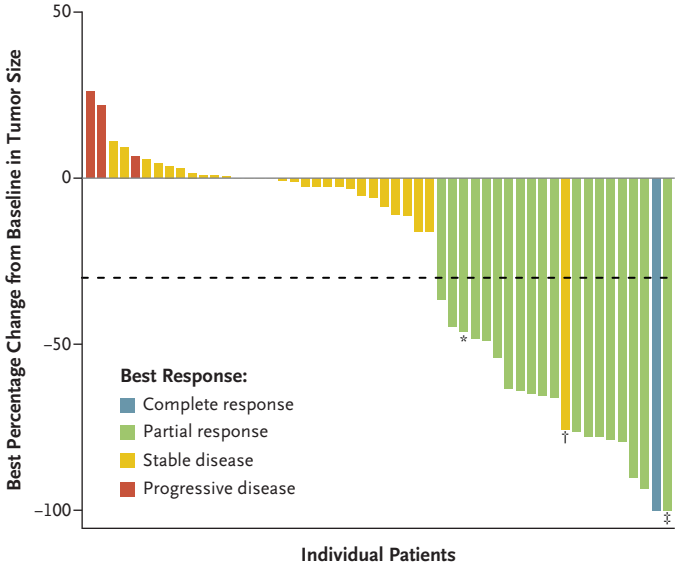


Figure 2. Best Target-Lesion Response.

The best percentage change from baseline in the target-lesion size is shown for each patient. The colors of the bars indicate the best response for each patient, and the dashed line represents a decrease of at least 30% in the target-lesion size. Patient 14 (asterisk) had an unconfirmed partial response. Patient 29 (dagger) had a partial response according to iRECIST but a best response of stable disease according to Response Evaluation Criteria in Solid Tumors, version 1.1, owing to an increase of more than 20% in target-lesion size before subsequent shrinkage (i.e., pseudoprogression). Patient 37 (double dagger) had a radiographic complete response of the target lesion, but bone abnormalities persisted.

immune partial response four cycles later. The other patient (Patient 34) had stable disease for more than 1 year before having a partial response after a bout of viral-induced vestibulitis.

SAFETY

Atezolizumab had an adverse-event profile consistent with those previously reported for atezolizumab monotherapy (Table S4). A total of 50 of 52 patients (96%) had a grade 1 or 2 adverse event; grade 3 adverse events that were considered by the investigators to be potentially related to atezolizumab were reported in 8 patients (15%). No treatment-related grade 4 or 5 events were reported. None of the patients discontinued treatment because of adverse events.

PHARMACODYNAMICS

Evaluable ASPS tumor-biopsy specimens that were obtained from patients with a best response of partial response (8 patients), stable disease (10 patients), or progressive disease (1 patient) were evaluated for required components of the mechanism of action of atezolizumab with the use of a multiplexed immunofluorescence assay (Fig. 4 and Tables S5 and S6). Expression of the molecular components, including PD-1 and PD-L1, was variable. The study was not powered to compare pharmacodynamic results across the three response groups; the results within each subgroup are described below.

In the partial-response group, analysis of pretreatment tumor-biopsy specimens showed six of six tumors positive for PD-L1, five of six positive for CD3+ lymphocytes expressing the receptor PD-1, six of six positive for CD8+ cytotoxic T lymphocytes (CTLs), and six of six with a regulatory T cell (Treg):CTL ratio of less than 1 — a ratio that indicates a permissive immune environment associated with tumor regression and overall-survival advantages in other cancers.[30] On cycle 3 day 1, tumor-biopsy specimens obtained from six of seven patients in the partial-response group were positive for all four mechanistic components, including one patient (Patient 37) whose tumor converted from negative to positive for PD-1–positive lymphocytes during atezolizumab therapy. The sole PD-L1–negative tumor in the partial-response group at cycle 3 (in Patient 40) converted to PD-L1–positive at cycle 6, on the basis of longitudinal assessments of circulating tumor cells (Fig. S2). At baseline, CD3ζ phosphorylation of the T-cell receptor, a biomarker of antigen recognition, was positive in four of six tumors, and the two other tumors (in Patients 1 and 43) converted from negative to positive by cycle 3 day 1. Unlike in many carcinomas,[31-33] activated CTLs were distributed throughout the tumor and stroma without exclusion at invasive margins, which indicated unimpeded movement within the tumor microenvironment.

In the stable-disease group, analysis of pretreatment biopsy specimens showed 9 of 10 tumors positive for PD-L1, 10 of 10 positive for intratumoral CTLs, and 10 of 10 with a favorable Treg:CTL ratio of less than 1. One patient (Patient 44) had a PD-L1–negative biopsy specimen, and PD-L1–negative status continued through the longer-term monitoring of circulating tumor cells into cycle 6. ASPS tumors in the stable-disease group were more likely to be negative for PD-1–expressing lymphocytes at baseline (7 of 10 patients) than those in the partial-response group

(1 of 6 patients), and the percentage of patients with a response was higher among patients with PD-1–positive lymphocytes in the tumor microenvironment before treatment (5 of 9 patients) than those without (1 of 8). Three patients in the stable-disease group had tumors that converted from PD-1–negative to PD-1–positive by cycle 3 day 1. As in the partial-response group, most evaluable biopsy specimens that were obtained before treatment and during treatment were positive for CD3ζ pY142; Patient 33 had a tumor that was negative before treatment but converted to positive by cycle 3 day 1.

Four patients with evaluable biopsy specimens had disease progression within five treatment cycles. The tumors in three of these four patients lacked both PD-L1 and PD-1 expression (Patient 44, stable disease), lacked PD-1 expression (Patient 41, stable disease), or had an unfavorably high Treg:CD8 ratio (Patient 30, progressive disease).

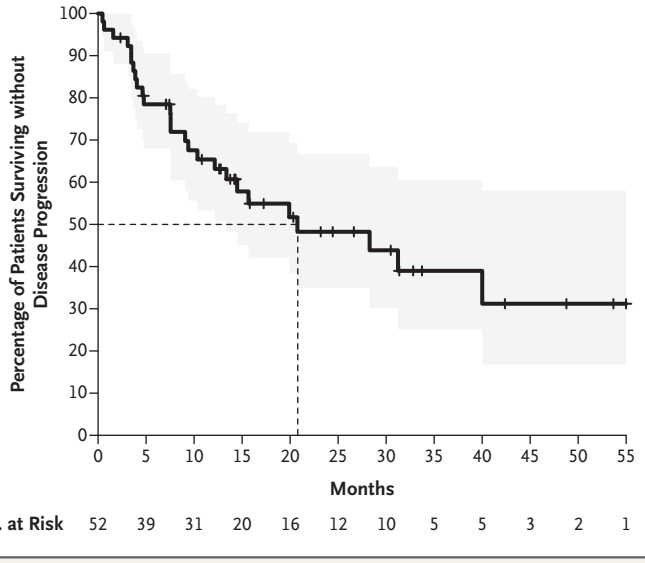


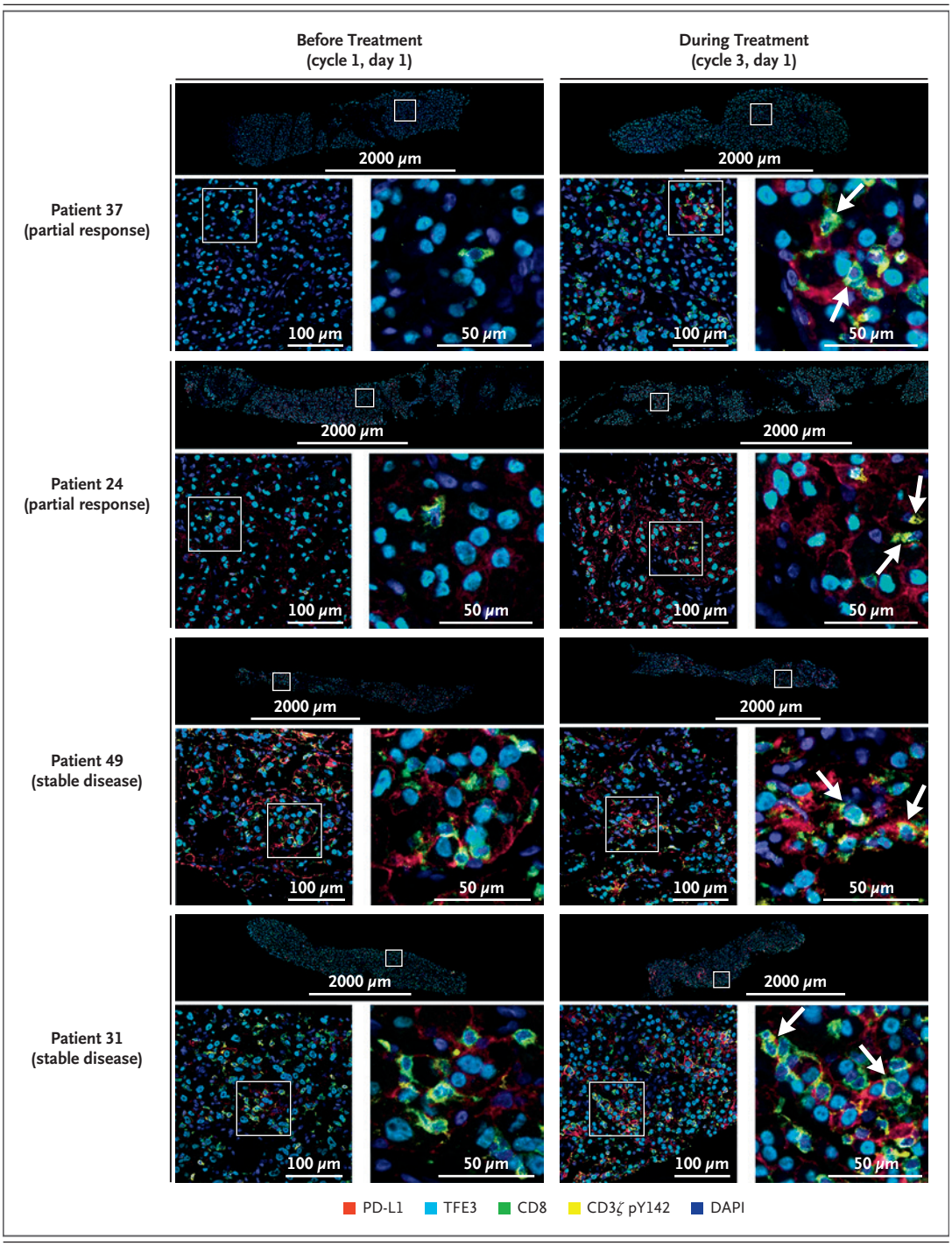
Figure 3. Kaplan–Meier Analysis of Progression-free Survival.

Kaplan–Meier estimates of progression-free survival are shown as of July 31, 2022. The dashed line indicates the median progression-free survival, and tick marks indicate unique censoring times. The gray-shaded areas indicate 95% pointwise confidence intervals, with standard errors obtained with the use of Greenwood's formula (computed on the basis of log[survival]).

DISCUSSION

This investigator-initiated phase 2 study showed robust, durable anticancer activity of atezolizumab in a diverse cohort of patients with advanced ASPS that reflects the population of patients with the disease. Objective responses according to RECIST, version 1.1 — including one complete response — occurred in approximately one third (37%) of the patients, many of whom had disease progression during previous systemic therapy. Responses were durable, and several were maintained for years after the final dose of atezolizumab. It should be noted that tumor assessments were performed less frequently in patients who remained in this study for more than 1 year, which potentially affected the assessment of progression-free survival results by 3 to 6 weeks. Still, the durations of response and progression-free survival achieved with atezolizumab therapy are uncommon with other therapies used in ASPS, even in contexts like this one in which patients may have been enrolled with indolent disease.[11,13,34,35] In the present study, tumor responses to atezolizumab typically occurred within the first 3 to 5 months, but three patients had received treatment for 1 year or longer before having a partial response. Late-onset responses to other immune checkpoint inhibitors have been reported in persons with ASPS.[10,11]

The results from previous trials evaluating immune checkpoint inhibitors in patients with various histologic types of cancer suggested that ASPS may be especially sensitive to immune checkpoint inhibition.[7,10,13,15,36] However, interpretation of those findings is limited by the small size of each ASPS cohort and, in some cases, the use of combined drug regimens. The present phase 2 study enrolled only patients with ASPS to evaluate the efficacy of atezolizumab monotherapy in this ultrarare histologic type. The incidence of response in this study closely resembles that observed among 10 patients with ASPS treated with combined PD-L1 and CTLA4 inhibitors.[36] However, the combination was associated with more frequent and more serious toxic effects than we observed with PD-L1 inhibition alone. This finding calls into question the value of adding CTLA4 inhibition to anti–PD-L1 treatment of ASPS. The combination of vascular endothelial growth factor (VEGF) inhibition and immune checkpoint blockade has also been studied in a small cohort of patients with ASPS, with 54.5% having a response.[10] On the basis of these encouraging results, we are currently evalu-



ating the addition of VEGF inhibition to PD-L1 blockade in patients who had disease progression during atezolizumab monotherapy in the present study. The incidence of response and immune landscapes that we observed with atezolizumab monotherapy suggest that the addition of a VEGF inhibitor may not be necessary in first-line treatment.

The pharmacodynamic data that are reported here show that many of the tumors harbored the molecular and cellular elements required for atezolizumab response (PD-1 and PD-L1, unhin-

Figure 4 (facing page). Pharmacodynamic Biomarkers in Tumor-Biopsy Specimens.

Multiplex immunofluorescence microscopy of core-needle tumor-biopsy specimens revealed the molecular target of atezolizumab (programmed death ligand 1 [PD-L1]–positive cells, red) and immune effector cells (CD8+ cytotoxic T lymphocytes [CTLs], green), dispersed among alveolar soft part sarcoma (ASPS) tumor cells expressing nuclear TFE3 (light blue). Some of these CTLs showed phosphorylation of the ζ chain of the T-cell receptor that is associated with antigen recognition (yellow, with example cells indicated by arrows). The nuclei of all TFE3-negative cells are dark blue from the DAPI (4′,6-diamidine-2-phenylindole) stain. Unlike in other cancers in which lymphocytes accumulate at tumor margins, CD8+ CTLs are widely dispersed into all areas of the ASPS tumor microenvironment in the four representative tumors shown here, which is consistent with unimpeded immune-cell migration. Patient 37, in the partial-response group, had increases in PD-L1 and CTL levels by a factor of 9 to 10 from the pretreatment baseline (left) to cycle 3 day 1 of treatment (right). Patient 24, in the partial-response group, and Patient 49, in the stable-disease group, had the persistent presence of PD-L1–positive cells and CTLs at baseline (left) and cycle 3 day 1 (right). Patient 31, in the stable-disease group, had both persistent and abundant PD-L1–positive cells and CTLs at baseline (left) and cycle 3 day 1 (right). The presence of molecular and cellular components that are required to respond to the mechanism of action of atezolizumab in nearly all the patients with evaluable tumor-biopsy specimens indicates that this cancer is primed to respond to immune checkpoint inhibitor therapy and is consistent with the high degree of clinical benefit conferred by atezolizumab therapy. Quantitation of each biomarker is presented in Table S5.

dered movement of CTLs with activated T-cell receptor, and a permissive tumor microenvironment) at baseline, whereas other tumors lacked one or more such components before treatment but converted to a responsive phenotype during atezolizumab administration. Given the possibility of conversion to an immunotherapy-responsive phenotype during treatment, patient selection based on pretreatment PD-1 or PD-L1 expression status may exclude patients with ASPS who have the potential to have a response to immune checkpoint inhibitor treatment. Adaptive induction of PD-L1 expression in tumor cells has also been observed in melanoma, in which tumor PD-L1 expression during treatment is more predictive of response to immune checkpoint inhibitors than expression at baseline.[37] Further study of the histologic breadth of this phenomenon, the molecular pathways involved, and the predictive power of biomarkers during early treatment is warranted.

Transcriptomic-profiling results indicate that ASPS tumors are among the most highly lymphocyte-infiltrated solid tumors in pediatric patients.[38] Using multiplex immunofluorescence analyses, we detected dense and unimpeded CTL tumor infiltrates in adults with ASPS. Furthermore, intratumoral CTLs with activated T-cell receptor (CD3ζ phosphorylated at tyrosine 142) were present by cycle 3 in all ASPS tumors with evaluable biopsy specimens, indicative of antigen recognition. The source and identity of these antigens remain unknown. We hypothesize that aberrant transcriptional activity of the characteristic type 1 and type 2 ASPL–TFE3 fusion proteins may serve as a substantial source of neoantigens in ASPS. Although type 1 and type 2 ASPL–TFE3 fusions differ in their transactivation properties,[4] descriptions of pathophysiological distinctions between the two fusion types are lacking. Even though the number of type 2 fusions in the present study was too small for a formal statistical analysis, our discovery that the *ASPL–TFE3* fusion types can be detected and distinguished with the use of venous blood samples should facilitate future assessments and investigation of the role of potential neoantigens derived from *ASPL–TFE3* fusions in tumor immunity.

A limitation of this study is that research biopsies — which were optional when the study began — were performed in only one of the patients whose initial response assessment was progressive disease. Specimens from several rapidly progressing tumors would have enabled comparison against responsive tumors for a better understanding of response or resistance mechanisms. Future analyses of specimens that were obtained from patients in this study at the time their disease progressed, before the start of atezolizumab–bevacizumab combination therapy, are expected to provide additional insight.

The results of this phase 2 clinical study, which formed the basis of the recent FDA approval, support the use of atezolizumab as a safe and effective treatment for advanced ASPS. Further investigation is needed to inform clinical decisions regarding the duration of atezolizumab treatment, the usefulness and appropriate timing of treatment breaks, and the potential benefit of atezolizumab rechallenge after disease progression.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

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REFERENCES

1. Stacchiotti S, Frezza AM, Blay J-Y, et al. Ultra-rare sarcomas: a consensus paper from the Connective Tissue Oncology Society community of experts on the incidence threshold and the list of entities. Cancer 2021;127:2934-42.
2. O'Sullivan Coyne G, Naqash AR, Sankaran H, Chen AP. Advances in the management of alveolar soft part sarcoma. Curr Probl Cancer 2021;45:100775.
3. Ladanyi M, Lui MY, Antonescu CR, et al. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene 2001;20:48-57.
4. Kobos R, Nagai M, Tsuda M, et al. Combining integrated genomics and functional genomics to dissect the biology of a cancer-associated, aberrant transcription factor, the ASPSCR1-TFE3 fusion oncoprotein. J Pathol 2013;229:743-54.
5. Williams A, Bartle G, Sumathi VP, et al. Detection of ASPL/TFE3 fusion transcripts and the TFE3 antigen in formalin-fixed, paraffin-embedded tissue in a series of 18 cases of alveolar soft part sarcoma: useful diagnostic tools in cases with unusual histological features. Virchows Arch 2011;458:291-300.
6. Food and Drug Administration Center for Drug Evaluation Research. Highlights of prescribing information for Tecentriq (atezolizumab) injection (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761034s047lbl.pdf).
7. Groisberg R, Hong DS, Behrang A, et al. Characteristics and outcomes of patients with advanced sarcoma enrolled in early phase immunotherapy trials. J Immunother Cancer 2017;5:100.
8. Groisberg R, Roszik J, Conley AP, et al. Genomics, morphoproteomics, and treatment patterns of patients with alveolar soft part sarcoma and response to multiple experimental therapies. Mol Cancer Ther 2020;19:1165-72.
9. Conley AP, Trinh VA, Zobniw CM, et al. Positive tumor response to combined checkpoint inhibitors in a patient with refractory alveolar soft part sarcoma: a case report. J Glob Oncol 2018;4:1-6.
10. 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.
11. 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 2, single-arm study. Clin Cancer Res 2020;26:6445-52.
12. Martin-Broto J, Hindi N, Grignani G, et al. Nivolumab and sunitinib combination in advanced soft tissue sarcomas: a multicenter, single-arm, phase Ib/II trial. J Immunother Cancer 2020;8(2):e001561.
13. 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 AcSe pembrolizumab study from Unicancer. J Clin Oncol 2021;39(15):Suppl:11520-.
14. Conry A, Peters M, Fried DB, et al. Complete response to dual immunotherapy in a young adult with metastatic alveolar soft part sarcoma enabled by a drug recovery program in a community practice. J Adolesc Young Adult Oncol 2020;9:449-52.
15. Yang J, Dong L, Yang S, et al. Safety and clinical efficacy of toripalimab, a PD-1 mAb, in patients with advanced or recurrent malignancies in a phase I study. Eur J Cancer 2020;130:182-92.
16. Mittal A, Arun Raj ST, Gupta A, Shamim SA, Barwad A, Rastogi S. Immune checkpoint inhibitors in alveolar soft part sarcoma: new standard of care? J Adolesc Young Adult Oncol 2021;10:501-2.
17. Mariuk-Jarema A, Koseła-Paterczyk H, Rogala P, et al. A durable complete response to immunotherapy in a patient with metastatic alveolar soft part sarcoma. Tumori 2020;106(6):NP9-NP13.
18. Su H, Yu C, Ma X, Song Q. Combined immunotherapy and targeted treatment for primary alveolar soft part sarcoma of the lung: case report and literature review. Invest New Drugs 2021;39:1411-8.
19. Dorman K, Burkhard-Meier A, Di Gioia D, et al. Treatment of metastatic alveolar soft part sarcoma with axitinib and

pembrolizumab in an 80-year-old patient with a history of autoimmune disorders. Anticancer Drugs 2023;34:311-6.
20. Xu Z, Zhang Y, Yu Y-H. Successful treatment of advanced alveolar soft part sarcoma with camrelizumab combined with apatinib: a case report. Ann Palliat Med 2021;10:785-92.
21. Palmeri M, Mehnert J, Silk AW, et al. Real-world application of tumor mutational burden-high (TMB-high) and microsatellite instability (MSI) confirms their utility as immunotherapy biomarkers. ESMO Open 2022;7:100336.
22. Chang W, Brohl AS, Patidar R, et al. Multidimensional clinOmics for precision therapy of children and adolescent young adults with relapsed and refractory cancer: a report from the Center for Cancer Research. Clin Cancer Res 2016;22:3810-20.
23. Lewin J, Davidson S, Anderson ND, et al. Response to immune checkpoint inhibition in two patients with alveolar soft-part sarcoma. Cancer Immunol Res 2018;6:1001-7.
24. Vander Jagt TA, Davis LE, Thakur MD, Franz C, Pollock JM. Pseudoprogression of CNS metastatic disease of alveolar soft part sarcoma during anti-PDL1 treatment. Radiol Case Rep 2018;13:882-5.
25. 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;45:228-47.
26. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 2017;18(3):e143-e152.
27. Herrick WG, Kilpatrick CL, Hollingshead MG, et al. Isoform- and phosphorylation-specific multiplexed quantitative pharmacodynamics of drugs targeting PI3K and MAPK signaling in xenograft models and clinical biopsies. Mol Cancer Ther 2021;20:749-60.
28. Srivastava AK, Navas T, Herrick WG, et al. Effective implementation of novel MET pharmacodynamic assays in translational studies. Ann Transl Med 2017;5:3.
29. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project. J Thorac Oncol 2018;13:1302-11.
30. Kareva I. Metabolism and gut microbiota in cancer immunoediting, CD8/Treg ratios, immune cell homeostasis, and cancer (immuno)therapy: concise review. Stem Cells 2019;37:1273-80.
31. Pai SI, Cesano A, Marincola FM. The paradox of cancer immune exclusion: immune oncology next frontier. Cancer Treat Res 2020;180:173-95.
32. Burandt E, Blessin NC, Rolschewski A-C, et al. T-cell density at the invasive margin and immune phenotypes predict outcome in vulvar squamous cell cancer. Cancers (Basel) 2022;14:4246.
33. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2020;17:139-52.
34. Stacchiotti S, Mir O, Le Cesne A, et al. Activity of pazopanib and trabectedin in advanced alveolar soft part sarcoma. Oncologist 2018;23:62-70.
35. Liu J, Fan Z, Li S, et al. Target therapy for metastatic alveolar soft part sarcoma: a retrospective study with 47 cases. Ann Transl Med 2020;8:1493.
36. Somaiah N, Conley AP, Parra ER, et al. Durvalumab plus tremelimumab in advanced or metastatic soft tissue and bone sarcomas: a single-centre phase 2 trial. Lancet Oncol 2022;23:1156-66.
37. Chen P-L, Roh W, Reuben A, et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. Cancer Discov 2016;6:827-37.
38. Brohl AS, Sindiri S, Wei JS, et al. Immuno-transcriptomic profiling of extracranial pediatric solid malignancies. Cell Rep 2021;37:110047.