Anlotinib plus TQB2450, a PD-L1 Antibody, in Patients with Advanced Alveolar Soft Part Sarcoma: A Single-Arm, Phase II Trial

Zhichao Tan[1], Yan Wu[2], Zhengfu Fan[1], Tian Gao[1], Wei Guo[3], Chujie Bai[1], Ruifeng Xue[1], Shu Li[1], Lu Zhang[1], Xinyu Wang[1], Ling Jia[2], and Jiayong Liu[1]

ABSTRACT

Purpose: Alveolar soft part sarcoma (ASPS) is an ultrarare soft-tissue sarcoma with a high rate of metastasis and no established treatment. This study aimed to explore the efficacy and safety of anlotinib (a tyrosine kinase inhibitor) and TQB2450 (a PD-L1 inhibitor) in patients with ASPS.

Patients and Methods: This single-arm, phase II study evaluated the efficacy of TQB2450, an anti–PD-L1 agent, combined with anlotinib, a tyrosine kinase inhibitor, in adults with advanced ASPS. TQB2450 was given intravenously (1,200 mg) on day 1, and anlotinib (12 mg/day) was taken orally from day 1 to 14 every 3 weeks. The primary endpoint was overall response rate, with secondary endpoints including duration of response, progression-free survival, and overall survival. Lymphocyte infiltration and tertiary lymphoid structure (TLS) were also analyzed as potential prognostic biomarkers.

Results: The study enrolled 29 patients, of whom 28 were evaluable (one withdrew because of acute pancreatitis). An objective response was achieved in 82.1% of patients, including 4 complete and 19 partial responses. The median time to response was 2.8 months, and the duration of response was not reached, with an estimated median progression-free survival of 35.2 months. Grade 3 to 4 treatment-related adverse events occurred in 44.8% of patients, with no study-related deaths. Responders had a higher proportion of TLS area, TLS density, and CD20-positive immune cells.

Conclusions: The combination of anlotinib and TQB2450 is effective and tolerable in patients with ASPS. TLS may serve as a prognostic biomarker, meriting further investigation.

Introduction

Alveolar soft part sarcoma (ASPS) is a rare tumor, which accounts for less than 1% of all soft-tissue sarcomas (STS). According to the Connective Tissue Oncology Society, ASPS is defined as ultrarare because of its very low incidence, with less than 1 case per one million people (1, 2). ASPS predominantly affects young adults, with a slight predominance in female (3). The defining molecular characteristic of ASPS is the unbalanced translocation of t(X;17)(p11;q25), which generates a chimeric fusion of *ASPL–TFE3* (4). The *ASPL–TFE3* fusion causes upregulation of angiogenesis via modulation of super-enhancer activity (5). Two distinct *ASPL–TFE3* fusion patterns have been reported, and alternative rearrangements have been described in a retrospective review, such as *HNRNPH3–TFE3*, *DVL2–TFE3*, and *PRCC–TFE3* gene fusions (6). However, to date, no clinical significance with respect to prognosis has been attributed to fusion type.

The management of ASPS typically involves surgical resection and/or systemic treatment for metastatic disease. As a highly malignant tumor, the disease presents with a metastatic rate of 60% to 70% at the time of diagnosis or later (7, 8). Moreover, it exhibits significant resistance to conventional chemotherapies, with a response rate lower than 10% (9, 10). The upregulation of angiogenic factors, including VEGF and hepatocyte growth factor receptor, has encouraged the exploration of targeted therapy, particularly tyrosine kinase inhibitors (TKI). A phase II randomized trial of sunitinib or cediranib in ASPS demonstrated an overall response rate (ORR) of 6.7% and 7.1% and a disease control rate (DCR) of 86.7% and 78.6%, respectively (11). In a pan-sarcoma phase II study, anlotinib monotherapy achieved an ORR of 46% in 13 patients with ASPS (12). Pazopanib, which was approved by the FDA for STSs, was confirmed effective in patients with ASPS by a retrospective study (13). Immune checkpoint inhibitors (ICI) represent a promising area of development in ASPS. A phase II basket trial, which aimed to evaluate the efficacy of pembrolizumab in rare and ultrarare sarcomas, showed an ORR of 57% in 14 patients with ASPS (14). Atezolizumab, a PD-L1 antagonist, also demonstrated encouraging and durable responses in ASPS, with an ORR of 37% (15). Based on this result from the study, the FDA approved atezolizumab for the treatment of unresectable or metastatic ASPS in 2022.

Because either TKI or ICI monotherapy has promising efficacy, co-administration of these two regimens is expected to exert a synergistic effect. In a pan-sarcoma, phase II clinical trial, axitinib plus pembrolizumab achieved good responses in the ASPS subgroup [ORR, 54.5%; median progression-free survival (PFS), 12.4 months; ref. 16]. Similarly, we conducted a single-arm, phase II clinical trial

[1]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Bone and Soft Tissue Tumor, Peking University Cancer Hospital and Institute, Beijing, China. [2]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Pathology, Peking University Cancer Hospital and Institute, Beijing, China. [3]Musculoskeletal Tumor Center, Peking University People's Hospital, Beijing, China.

Z. Tan, Y. Wu, and Z. Fan contributed equally to this article.

Corresponding Author: Jiayong Liu, Peking University Cancer Hospital and Institute, Fucheng Road 54th, Haidian District, Beijing 100142, China. E-mail: liujiayong_doc@163.com

Clin Cancer Res 2024;30:5577–83

doi: 10.1158/1078-0432.CCR-24-2444

©2024 American Association for Cancer Research

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

Translational Relevance

Alveolar soft part sarcoma (ASPS) is an ultrarare, highly malignant tumor with a limited response to conventional therapies. This single-arm, phase II study investigates the combination of anlotinib, a multiple tyrosine kinase inhibitor, and TQB2450, a PD-L1 inhibitor, to explore its efficacy and safety in treating advanced ASPS. This combination therapy achieved a high objective response rate (82.1%) and prolonged progression-free survival (35.2 months), demonstrating a higher response rate compared with historical data on anlotinib alone. Notably, the study identifies tertiary lymphoid structure density as a potential predictive biomarker for treatment response, underscoring the importance of the tumor immune microenvironment in ASPS. These findings provide a promising new therapeutic approach and highlight the potential of tertiary lymphoid structures as a prognostic factor, paving the way for future studies to optimize treatment strategies in ASPS and potentially other sarcomas.

in the pan-sarcoma group, to assess the potency of TQB2450 and anlotinib. A total of 12 patients with ASPS were included in this study. For the evaluable patients, the ORR was 75% and the median PFS was 23.06 months (2). However, these studies were limited by small sample sizes, and predictive factors for responses were unclear. Tertiary lymphoid structures (TLS) have been discovered to be associated with improved outcomes in patients with STS (17, 18). Hence, we conducted this expansion trial, to evaluate the efficacy of TQB2450 and anlotinib for ASPS and to investigate the hypothesis that TLS can serve as a predictive factor for treatment response in patients with ASPS.

Patients and Methods

Study design and patients

This was an expanded single-arm, phase II trial of TQB2450-Ib-02 (Chinadrugtrials.org.cn identifier: CTR20190938) in patients with advanced ASPS. The study was conducted at Peking University Cancer Hospital and Institute and Peking University People's Hospital in China. The study was conducted in accordance with the Declaration of Helsinki and approved by the Peking University Cancer Hospital and Institute Ethics Committee. All patients provided written informed consent. The authors vouch for the accuracy and completeness of the data.

Eligible patients were 18 years of age or older, with histologically confirmed ASPS (including newly diagnosed, unresectable, or metastatic cases and measurable disease with clinically confirmed disease progression). Key exclusion criteria included prior treatment with antiangiogenic therapy or immunotherapy and patients with central nervous system metastasis. In this study, participants were enrolled without specific limitations based on sex/gender. The sex/gender of participants was recorded during enrollment for descriptive purposes and potential exploratory analysis.

Treatment

Anlotinib (12 mg/day) was administered orally from day 1 to 14 every 3 weeks. TQB2450 was administered by intravenous infusion at a dosage of 1,200 mg on day 1 every 3 weeks for 60 ± 5 minutes. The combined treatment would be continued until disease progression, unacceptable toxicity, or patient withdrawal of consent. Details about the dose interruption and treatment discontinuation were described in the previous article (2). Patients with progressive disease for the first time, identified according to RECIST version 1.1, continued to receive the treatment and were evaluated according to the immune RECIST (iRECIST) criteria. Randomization was not used as this was a single-arm study.

Endpoints and assessments

Tumor assessments were performed according to RECIST version 1.1 at baseline, every two cycles for the first year, and every three cycles after the first year. Tumors were measured using CT or MRI. Confirmatory scans were performed at least 4 weeks after initial documentation of objective response. Efficacy was further confirmed with iRECIST; that is, patients who had disease progression according to RECIST 1.1 were further examined to confirm the same using iRECIST.

The primary endpoint was the objective response rate (ORR), which was defined as the percentage of patients with a complete response (CR) or partial response (PR). The secondary endpoints included PFS, overall survival (OS), DCR, and adverse effects (AE) during the study. AEs were reported according to NCI Common Terminology Criteria for Adverse Events v5.0. PFS was defined as the time from the first date of drug administration to the time of disease progression according to RECIST 1.1 or death due to any cause, whichever occurred first. OS was measured from the first date of drug administration to the date of death due to any cause. Patients who were event-free or were lost to follow-up were censored at the time of the last visit.

Multiplex immunofluorescence and IHC analyses

We carried out multiplex immunofluorescence (mIF) staining with a seven-color fluorescence IHC kit (Beijing PhenoVision Bio Co., Ltd) according to the manufacturer's instructions. Four-micron-thick tissue sections cut from formalin-fixed, paraffin-embedded blocks of ASPS underwent deparaffinization, rehydration, and antigen retrieval with pH 6.0 citrate buffer. Sections were then treated with hydrogen peroxide and blocking reagents for 10 minutes each. Primary antibodies, including CD3 (Abcam Cat. # AC-0004, RRID: AB_11000899), CD4 (Abcam Cat. # 3205-1, RRID: AB_2073216), Foxp3 (Abcam Cat. # 3100-1, RRID: AB_2104897), CD8 (Abcam Cat. # 4207-1, RRID: AB_764503), CD20 (Abcam Cat. # AP-0012, RRID: AB_10703983), CD21 (Abcam Cat. # 2546-1, RRID: AB_1267035), CD23 (Abcam Cat. # 2685-1, RRID: AB_2103171), CD68 (Abcam Cat. # 2135-1, RRID: AB_991703), CD163 (Abcam Cat. # 3659-1, RRID: AB_10703793), PD-1 (Abcam Cat. # AB201825, RRID: AB_2728811), MUM-1 (Abcam Cat. # ab12501, RRID: AB_2296152), and TFE3 (Abcam Cat. # ab70008, RRID: AB_1271208), were applied for 30 minutes at room temperature. The signals were then detected using the PVB anti-Rb/Mm-HRP secondary antibody followed by PVB tyramide signal amplification fluorophores for 10 minutes. Sections were counterstained with 4'6-diamidino-2-phenylindole (DAPI) and mounted.

The stained sections were scanned at 200× magnification using the PhenoImager HT system (Akoya Biosciences) and analyzed using a Pheno whole slide image analysis system built and trained from the Oncotopix Discovery system (Visiopharm), version 4.5.6.5. We identified and staged TLSs based on the presence of specific cellular markers (CD20, CD21, and CD23). The tumor area of ASPS was automatically calculated based on the staining of TFE3. The density and area percentage of TLS were calculated as TLS

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

number/tumor area and sum of TLS area/tumor area, respectively. Mature TLSs were identified by the presence of a network of CD23-positive dendritic cells (DC) or a germinal center, whereas primary TLSs were identified by displaying no germinal center and no network of CD23-positive DCs on immunofluorescence.

Additionally, PD-L1 expression was assessed using the PD-L1 22C3 pharmDx (Agilent Cat. # SK006, RRID: AB_2889976) and evaluated using combined positive scores (CPS).

Statistical analysis

In a previous multi-institutional phase II study evaluating the efficacy of anlotinib in STS, the incidence of response was 46% in 13 ASPS participants (12). Assuming that the true incidence of response would reach 70%, we estimated that enrollment of 25 evaluable patients would provide 90% power to reject a null hypothesis of an incidence of response of 40%, using a single-stage clinical trial design with a one-sided 5% significance level.

All eligible patients who received at least one dose of the study medication were included in the analysis of the incidence of response. All statistical analyses were performed using Statistical Analysis System version 9.4 (RRID: SCR_008567). Clinical and demographic characteristics of the study participants were summarized using descriptive statistics. The incidence of AEs is expressed in percentage. ORR and DCR are expressed as percentages with their 95% confidence intervals (CI) calculated by the Clopper–Pearson method. PFS and OS were estimated using the Kaplan–Meier survival method, and their 95% CIs were estimated by the Brookmeyer–Crowley method. For other variables such as demographic data, continuous variables are represented as mean ± SD or median and IQR, and categorical variables are represented as percentages. Among-group comparisons of numerical variables were calculated using a two-tailed Mann–Whitney test ($P < 0.05$).

Blinding was implemented at the level of the outcome assessors. Specifically, the individuals responsible for evaluating the outcomes were not informed of the specific hypotheses under investigation or the expected effects of the intervention. Additionally, any coding or data entry processes were designed to prevent the assessors from being influenced by participant information. This approach was taken to reduce the risk of bias in outcome measurement.

Data availability

This was an expanded single-arm, phase II trial of TQB2450-Ib-02 (Chinadrugtrials.org.cn identifier: CTR20190938) in patients with advanced ASPS. The Supplementary Material contains the study protocol. Because the trial protocol did not include provisions for data sharing, the trial data will not be publicly disclosed. However, data can be made available upon specific request to the corresponding author. Such requests will be evaluated and approved by the investigator and collaborator. Once approved, data sharing will be facilitated through a secure online platform, contingent upon the signing of a data access agreement.

Results

Patient characteristics

From January 2019 to January 2021, a total of 29 patients with ASPS were enrolled at two institutions. The demographic characteristics of these patients are summarized in **Table 1**. The median age was 29 years (range, 19–46 years), with a slightly male predominance of 51.7%. A subset of patients (20.7%) had undergone prior lines of chemotherapy. The most common metastatic site was the lung (100.0%), followed by the bone (10.3%), liver (6.8%), and brain (3.4%).

Table 1. Demographic and clinical characteristics of evaluable patients at baseline.

Demographic	Study population (*N* = 29)
Age, median (range)	29.0 (19–46)
Gender, *n* (%)	
Male	15 (51.7)
Female	14 (48.3)
Previous chemotherapy, *n* (%)	
Yes	6 (20.7)
No	23 (79.3)
Disease stage, *n* (%)	
Locally advanced	0 (0)
Metastatic	29 (100)
Metastatic site, *n* (%)	
Lung	29 (100)
Bone	3 (10.3)
Liver	2 (6.8)
Brain	1 (3.4)

Clinical efficacy

At the data cutoff date of January 31, 2024, among the 28 patients evaluable for efficacy (1 withdrew from the study), an objective response occurred in 23 (82.1%; 95% CI, 63.1%–93.9%), with 19 patients having a confirmed PR and 4 patients having a confirmed CR as their best response (**Fig. 1**). The response was rapid and long-term sustained. Among the 23 patients with a confirmed response (according to RECIST v1.1), the median time to response was 2.8 months (range, 1.2–11.0 months; **Fig. 2**; Supplementary Fig. S1). The duration of response was not reached. Three patients (two achieved CR and one achieved PR) took a break, which lasted 37.2, 28.6, and 8.6 months, respectively, with no relapse observed.

The median follow-up time was 23.9 months (95% CI, 19.6–30.1). At the data cutoff date, 11 (37.9%) patients discontinued treatment because of disease progression. The estimated median PFS was 35.2 months, and the PFS rates at 6 and 12 months were 89.3% and 78.4%, respectively (**Fig. 3**). No death occurred during the follow-up, and the median OS was not reached.

Safety

The AE profile was consistent with the previously reported, pan-sarcoma profile (2). All patients experienced treatment-related AEs (TRAE, incidence of 100%). The most common TRAEs of any grade were hypothyroidism (89.7%), hypertriglyceridemia (86.2%), elevated blood lactate dehydrogenase (79.3%), hyperuricemia (72.4%), and hypercholesterolemia (72.4%). Grade 3 to 4 TRAEs were documented in 13 (44.8%) patients. The most common grade 3 to 4 TRAEs were hypertriglyceridemia (13.8%), elevated lipase (6.9%), and proteinuria (6.9%). Dose reduction of anlotinib was performed in three patients owing to consecutive grade 2 to 3 proteinuria. TRAEs led to treatment termination of TQB2450 in one patient with immune-related acute pancreatitis. No grade 5 TRAE was reported (Supplementary Table S1).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

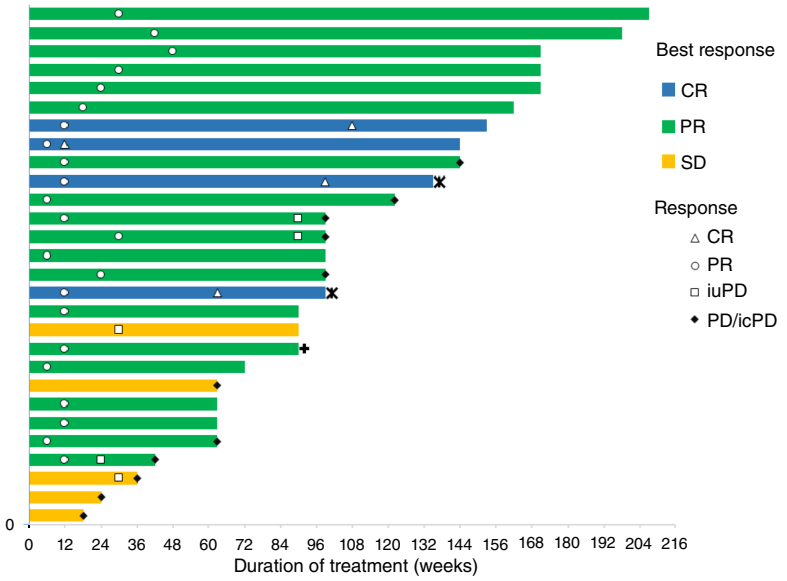

Figure 1.
Patient response to anlotinib and TQB2450. Time in the study (in weeks) is shown for each patient. The colors of the bars indicate the best response for each patient, and the time to the first CR or PR is indicated by a triangle or circle, respectively. The time to iuPD or PD/icPD is indicated by a hollow square or a filled square, respectively. iuPD or icPD was evaluated by iRECIST criteria. iuPD was defined as an increase in the sum of all TLs by at least 20% (but at least ≥5 mm) compared with the time point with the lowest TL sum, or an unequivocal progression of non-TL, or by the occurrence of new measurable and/or non-measurable TLs. icPD was defined as a further progress of the TL or non-TL after 6 weeks of the diagnosis of iuPD. One patient (cross) had a PR, but the remaining lesion was osteolytic; two patients (asterisk) had a CR. These three patients (cross and asterisk) took a break, with no relapse until date. icPD, immune-confirmed progressive disease; iuPD, immune-unconfirmed progressive disease; PD, progressive disease; SD, stable disease; TL, target lesion.

Exploration of the immune microenvironment

We obtained specimens from seven patients, of whom three patients were classified as achieving CR or PR, whereas the remaining four patients exhibited no response and were categorized as progressive disease. Through mIF analysis, we observed a notable distinction, approaching statistical significance, in the density and area percentage of TLS between these two groups. Good responders showed a median TLS density of 0.105 per square millimeter and an area percentage of 0.494%, compared with 0.012 per square millimeter and 0.034% area percentage in poor responders ($P = 0.057$ for both comparisons; **Fig. 4**). In our cohort, mature TLS was identified exclusively in one good responder (case 2), whereas the TLSs observed in the remaining cases were classified as primary. Nevertheless, our analysis revealed no significant correlation between the maturation status of TLS and treatment response. Furthermore, CD20-positive cell percentages indicated a trend toward higher values in good responders (1.33% vs. 0.19%; $P = 0.057$; **Fig. 4**), whereas the remaining immune cells showed no significant differences. The results of TLS and immune cell infiltration are presented in Supplementary Tables S2 and S3.

PD-L1 expression varied significantly within the cohort, with a high expression (CPS of 70) in one poor responder, whereas the highest CPS in good responders was only 5, with remaining cases below 1 (Supplementary Fig. S2).

Discussion

To the best of our knowledge, this investigator-initiated phase II trial is the largest prospective study with regard to the antitumor activity of the combination of TKI and immune checkpoint blockade in the specific cohort of ASPS. Objective responses were documented in 82.1% patients (including 4 clinically confirmed CR), which statistically exceeded the historical benchmark of TKI monotherapy (46%). Tumor responses occurred rapidly (typically within 2–6 months) and were durable.

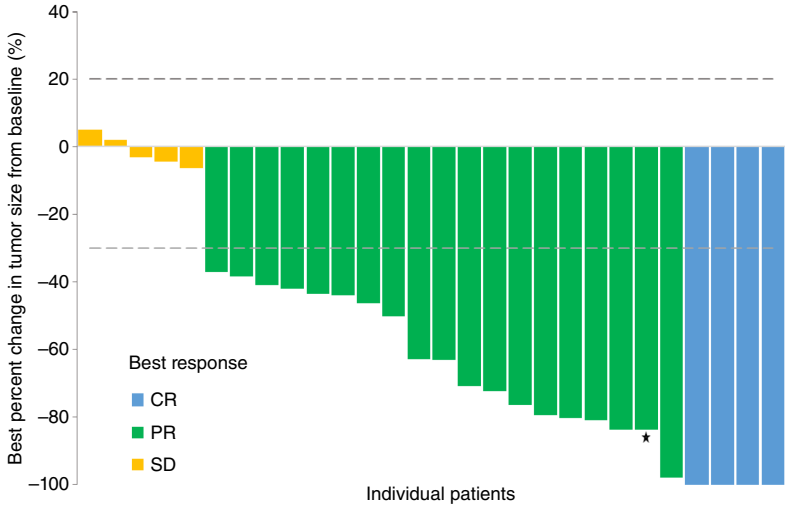

Figure 2.
Best target lesion response. The best percent change in the target-lesion size from baseline is shown for each patient. The colors of the bars indicate the best response for each patient, and the dashed line represents a decrease of 30% or an increase of 20% in the target lesion, respectively. Patient 31 (star) had a radiographic PR, with a residual target pulmonary nodule <3 mm, and all nontarget lesions disappeared for over 1 year. SD, stable disease.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

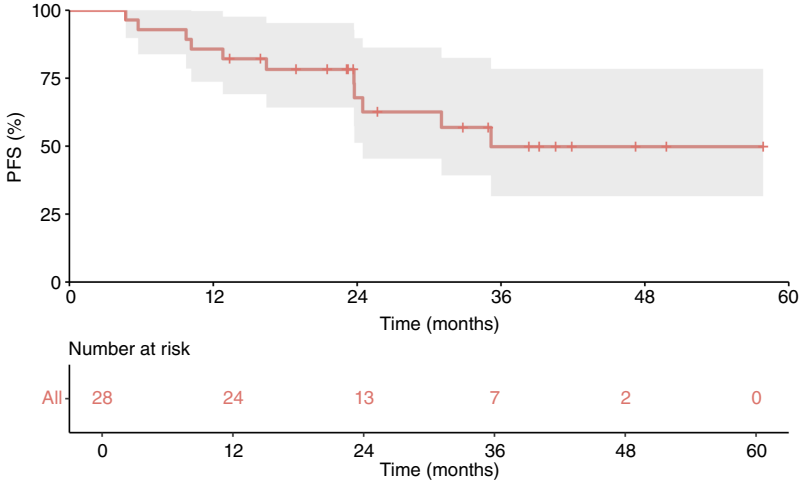

Figure 3.
PFS using Kaplan–Meier analysis.

The reliability of VEGF inhibitors on ASPS was well documented, with a median PFS ranging from 5.5 to 24.5 months with different broad-spectrum TKIs (11, 13, 19–22). Besides, since the discovery of ICIs, a series of studies demonstrated that ASPS might be especially sensitive to PD-1/PD-L1 blockade (23–26). A phase II prospective study evaluating the efficacy of atezolizumab, a PD-L1 antagonist, reported an ORR of 37% and a median PFS of 20.8 months in 52 patients with ASPS (15), further supporting the contribution of ICIs. The combination of VEGF inhibition and immune checkpoint blockade was explored in small cohorts of patients with ASPS, with a response rate of 54.5% in the axitinib–pembrolizumab study and 50% in the sunitinib–nivolumab study (16, 27), but the necessity of the combination remained unclear. Several previous studies reported that inhibition of VEGF function can lower immunosuppression and

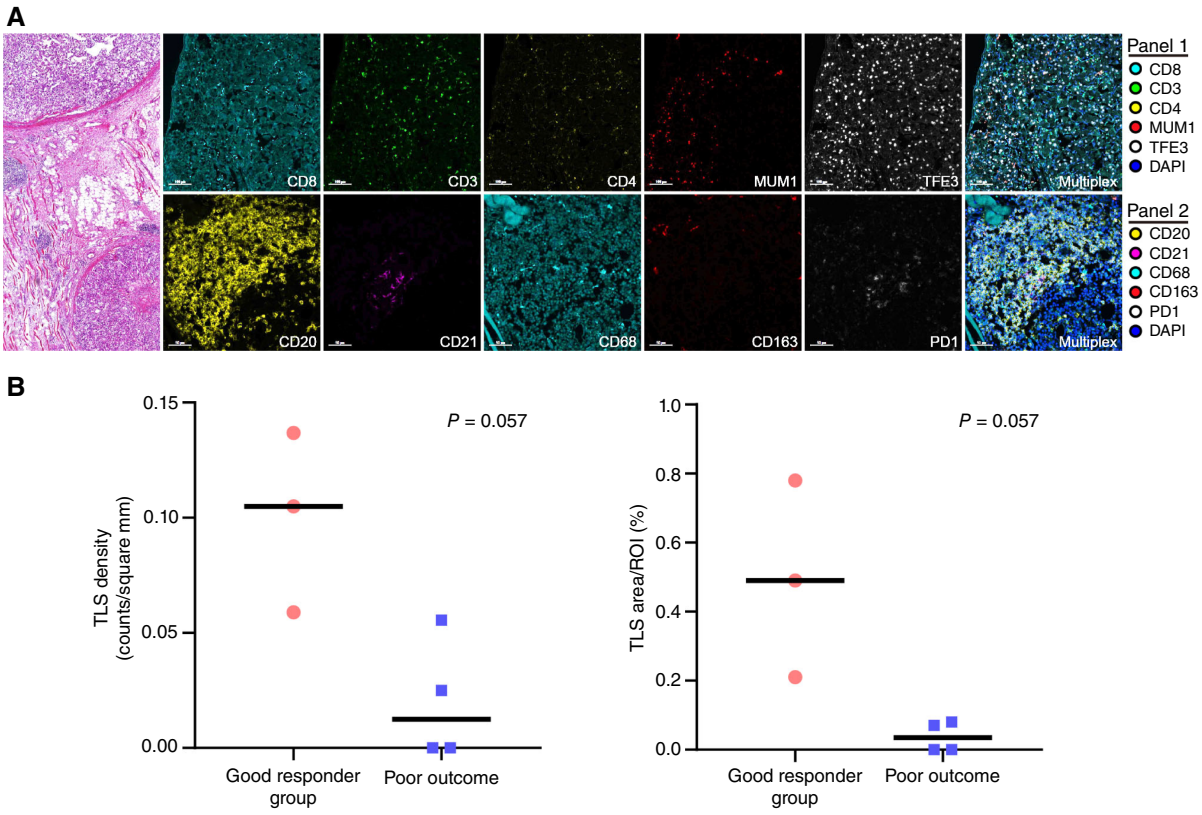

Figure 4.
Staining of immune markers in ASPS using mIF. **A,** Representative images of mIF staining on ICs and TLSs in a good responder (case 2). **B,** Comparison of the CD20 cell percentage, TLS density, and TLS area percentage between good and poor responders. IC, immune cells; ROI, region of interest.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

improve the efficacy of ICI therapy by increasing $CD8^+$ T-cell infiltration, preventing the inhibition of DC maturation, and regulating the expansion of myeloid-derived suppressor cells and regulatory T cells (28–32). In our study, the incidence of response and median PFS were significantly superior to those of anlotinib or atezolizumab monotherapy (12, 15), which advocated the synergistic effect of TKIs with ICIs.

The AEs were generally tolerable. Grade 3/4 TRAEs occurred in 44.8% patients but mainly were hypertriglyceridemia, elevated lipase, and proteinuria, most of which could be resumed after adjusting medication dose and symptomatic treatment. The toxicity profile was consistent with previous clinical trials of TKIs plus PD-1 inhibitors, such as anlotinib plus TQB2450 in the pan-sarcoma cohort (36.67%) and axitinib plus pembrolizumab (39%) in the ASPS cohort (2, 16).

The molecular mechanisms underlying the responses to ICIs remained unclear in ASPS. Tumor mutational burden and microsatellite instability, conventionally regarded as a response biomarker for ICIs in many other malignant tumors, are reported to be low in ASPS (33–35). Using mIF analyses to examine the immune microenvironment of ASPS, we observed a correlation between the density and area percentage of TLSs, as well as CD20-positive cell percentages and treatment outcomes. TLS serves as a site for priming and activation of antitumor immune responses, and their presence has been associated with high response to PD-1 blockade and improved survival in various ICI-treated tumors, including STSs (17, 18, 36–40). Our observation of higher TLS density and area percentage in good responders aligns with these findings, suggesting that TLS presence will serve as a predictor for ICI response in patients with ASPS. Furthermore, the differential expression of CD20-positive cells points toward the potential role of B cells within the TLS in mediating effective antitumor responses, a notion supported by recent studies highlighting the contribution of B cells to the efficacy of immunotherapy. In addition, a retrospective study of multitype cohorts of cancer, though STSs were not included, showed that the presence of mature TLS was associated with better ORR, PFS, and OS, independent of PD-L1 expression and $CD8^+$ T-cell density (40). However, our study did not observe a correlation between the maturation status of TLS and therapeutic response. This could be attributed to the limited number of cases within our cohort, coupled with the predominance of early-stage TLS in all but one patient, precluding the possibility of further subgroup comparisons. The paradox of elevated PD-L1 expression in a poor responder underscores the complexity of PD-L1 as an isolated predictive marker. PD-1/PD-L1 expression is critical in various solid tumors to guide anti–PD-1 treatment but is indecisive in ASPS (33, 35). The adaptive induction of PD-L1 expression in tumor cells during anti–PD-L1 treatment has been reported to predict ICI responses (15), but a decisive biomarker before ICI treatment is imperative for patients with ASPS. The implications of TLS presence and PD-L1 expression in forecasting the immunotherapeutic response for ASPS, and potentially across a spectrum of sarcomas, necessitate further investigation within an expanded cohort.

There are several important limitations to our study. First, the biopsies, which were not mandatory when the study began, were collected only from a limited subset of patients. These specimens may not represent the whole scenario of the whole cohort, inevitably causing bias. Second, we lacked specimens after treatment, which may have enabled our comparison between responsive and irresponsive tumors for a comprehensive understanding of resistance mechanisms. Third, it should be emphasized that the study was a single-arm study. A randomized study is expected to confirm that the combination of TKIs and ICIs is superior to either drug alone.

Conclusion

The results of this phase II clinical study support the combination use of anlotinib and PD-L1 inhibitor as an effective and tolerable treatment in patients with ASPS, and TLS density might be a promising prognostic factor. However, as a single-arm study, the necessity to add VEGF inhibitors to ICIs in ASPS is still unclear. Further exploration is needed to investigate whether the VEGF inhibitors exert a synergistic or an additive effect to ICIs.

Authors' Disclosures

Z. Tan reports nonfinancial support from Chiatai Tianqing Pharmaceutical Group Co., Ltd. during the conduct of the study. J. Liu reports nonfinancial support from Chiatai Tianqing Pharmaceutical Group Co., Ltd. during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

Z. Tan: Conceptualization, data curation, writing–original draft. **Y. Wu:** Investigation, writing–original draft. **Z. Fan:** Project administration. **T. Gao:** Supervision. **W. Guo:** Project administration. **C. Bai:** Project administration. **R. Xue:** Data curation. **S. Li:** Investigation. **L. Zhang:** Project administration. **X. Wang:** Project administration. **L. Jia:** Investigation, visualization. **J. Liu:** Conceptualization, supervision, funding acquisition, methodology, writing–review and editing.

Acknowledgments

All authors have read the authorship policy for the journal. We are grateful to all the patients who generously volunteered to participate in this study. We thank the research nurses and study coordinators for their efforts on behalf of the patients. We thank Chiatai Tianqing Pharmaceutical Group Co., Ltd. for providing the drug for this clinical trial. This work was supported by the Wu Jieping Medical Foundation (320.6750.2021-17-12).

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received July 30, 2024; revised August 22, 2024; accepted October 23, 2024; published first October 25, 2024.

References

1. Stacchiotti S, Frezza AM, Blay J-Y, Baldini EH, Bonvalot S, Bovée JVMG, 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. Liu J, Gao T, Tan Z, Li S, Xu J, Bai C, et al. Phase II study of TQB2450, a novel PD-L1 antibody, in combination with anlotinib in patients with locally advanced or metastatic soft tissue sarcoma. Clin Cancer Res 2022;28:3473–9.
3. Paoluzzi L, Maki RG. Diagnosis, prognosis, and treatment of alveolar soft-part sarcoma: a review. JAMA Oncol 2019;5:254–60.
4. Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, 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.
5. Tanaka M, Chuaychob S, Homme M, Yamazaki Y, Lyu R, Yamashita K, et al. ASPSCR1::TFE3 orchestrates the angiogenic program of alveolar soft part sarcoma. Nat Commun 2023;14:1957.
6. Dickson BC, Chung CT-S, Hurlbut DJ, Marrano P, Shago M, Sung Y-S, et al. Genetic diversity in alveolar soft part sarcoma: a subset contain variant fusion

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025

genes, highlighting broader molecular kinship with other MiT family tumors. Genes Chromosomes Cancer 2020;59:23–9.
7. Hagerty BL, Aversa J, Diggs LP, Dominguez DA, Ayabe RI, Blakely AM, et al. Characterization of alveolar soft part sarcoma using a large national database. Surgery 2020;168:825–30.
8. Kayton ML, Meyers P, Wexler LH, Gerald WL, LaQuaglia MP. Clinical presentation, treatment, and outcome of alveolar soft part sarcoma in children, adolescents, and young adults. J Pediatr Surg 2006;41:187–93.
9. Portera CA Jr, Ho V, Patel SR, Hunt KK, Feig BW, Respondek PM, et al. Alveolar soft part sarcoma: clinical course and patterns of metastasis in 70 patients treated at a single institution. Cancer 2001;91:585–91.
10. Reichardt P, Lindner T, Pink D, Thuss-Patience PC, Kretzschmar A, Dörken B. Chemotherapy in alveolar soft part sarcomas. What do we know? Eur J Cancer 2003;39:1511–6.
11. Nguyen J, Takebe N, Kummar S, Razak A, Chawla SP, George S, et al. Randomized phase II trial of sunitinib or cediranib in alveolar soft part sarcoma. Clin Cancer Res 2023;29:1200–8.
12. Chi Y, Fang Z, Hong X, Yao Y, Sun P, Wang G, et al. Safety and efficacy of anlotinib, a multikinase angiogenesis inhibitor, in patients with refractory metastatic soft-tissue sarcoma. Clin Cancer Res 2018;24:5233–8.
13. Stacchiotti S, Mir O, Le Cesne A, Vincenzi B, Fedenko A, Maki RG, et al. Activity of pazopanib and trabectedin in advanced alveolar soft part sarcoma. Oncologist 2018;23:62–70.
14. Blay J-Y, Chevret S, Le Cesne A, Brahmi M, Penel N, Cousin S, et al. Pembrolizumab in patients with rare and ultra-rare sarcomas (AcSé pembrolizumab): analysis of a subgroup from a non-randomised, open-label, phase 2, basket trial. Lancet Oncol 2023;24:892–902.
15. Chen AP, Sharon E, O'Sullivan-Coyne G, Moore N, Foster JC, Hu JS, et al. Atezolizumab for advanced alveolar soft part sarcoma. N Engl J Med 2023;389:911–21.
16. Wilky BA, Trucco MM, Subhawong TK, Florou V, Park W, Kwon D, 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.
17. Italiano A, Bessede A, Pulido M, Bompas E, Piperno-Neumann S, Chevreau C, et al. Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort. Nat Med 2022;28:1199–206.
18. Petitprez F, de Reyniès A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature 2020;577:556–60.
19. Oh CR, Hong JY, Kim JH, Lee JS, Kim HS, Kim TW, et al. Real-world outcomes of pazopanib treatment in Korean patients with advanced soft tissue sarcoma: a multicenter retrospective cohort study. Target Oncol 2020;15:485–93.
20. Judson I, Morden JP, Kilburn L, Leahy M, Benson C, Bhadri V, et al. Cediranib in patients with alveolar soft-part sarcoma (CASPS): a double-blind, placebo-controlled, randomised, phase 2 trial. Lancet Oncol 2019;20:1023–34.
21. Stacchiotti S, Negri T, Zaffaroni N, Palassini E, Morosi C, Brich S, et al. Sunitinib in advanced alveolar soft part sarcoma: evidence of a direct antitumor effect. Ann Oncol 2011;22:1682–90.
22. Kim M, Kim TM, Keam B, Kim YJ, Paeng JC, Moon KC, et al. A phase II trial of pazopanib in patients with metastatic alveolar soft part sarcoma. Oncologist 2019;24:e20–9.
23. Groisberg R, Hong DS, Behrang A, Hess K, Janku F, Piha-Paul S, et al. Characteristics and outcomes of patients with advanced sarcoma enrolled in early phase immunotherapy trials. J Immunother Cancer 2017;5:100.
24. Liu J, Fan Z, Bai C, Li S, Xue R, Gao T, et al. Real-world experience with pembrolizumab in patients with advanced soft tissue sarcoma. Ann Transl Med 2021;9:339.
25. Yang J, Dong L, Yang S, Han X, Han Y, Jiang 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.
26. Blay J-Y, Penel N, Ray-Coquard IL, Cousin S, Bertucci F, Bompas E, et al. High clinical activity of pembrolizumab in chordoma, alveolar soft part sarcoma (ASPS) and other rare sarcoma histotypes: the French AcSÃ© pembrolizumab study from Unicancer. J Clin Oncol 2021;39(Suppl 15):11520.
27. Martin-Broto J, Hindi N, Grignani G, Martinez-Trufero J, Redondo A, Valverde C, et al. Nivolumab and sunitinib combination in advanced soft tissue sarcomas: a multicenter, single-arm, phase Ib/II trial. J Immunother Cancer 2020;8:e001561.
28. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci U S A 2012;109:17561–6.
29. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2:1096–103.
30. Mimura K, Kono K, Takahashi A, Kawaguchi Y, Fujii H. Vascular endothelial growth factor inhibits the function of human mature dendritic cells mediated by VEGF receptor-2. Cancer Immunol Immunother 2007;56:761–70.
31. Finke J, Ko J, Rini B, Rayman P, Ireland J, Cohen P. MDSC as a mechanism of tumor escape from sunitinib mediated anti-angiogenic therapy. Int Immunopharmacol 2011;11:856–61.
32. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 2013;73:539–49.
33. Lewin J, Davidson S, Anderson ND, Lau BY, Kelly J, Tabori U, et al. Response to immune checkpoint inhibition in two patients with alveolar soft-part sarcoma. Cancer Immunol Res 2018;6:1001–7.
34. Groisberg R, Roszik J, Conley AP, Lazar AJ, Portal DE, Hong DS, 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.
35. Shi Y, Cai Q, Jiang Y, Huang G, Bi M, Wang B, 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.
36. Schumacher TN, Thommen DS. Tertiary lymphoid structures in cancer. Science 2022;375:eabf9419.
37. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer 2019;19:307–25.
38. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature 2020;577:549–55.
39. Fridman WH, Meylan M, Petitprez F, Sun C-M, Italiano A, Sautès-Fridman C. B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat Rev Clin Oncol 2022;19:441–57.
40. Vanhersecke L, Brunet M, Guégan J-P, Rey C, Bougouin A, Cousin S, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nat Cancer 2021;2:794–802.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/30/24/5577/3524279/ccr-24-2444.pdf by Boston University user on 11 February 2025