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Research article

Efficacy and toxicity of anlotinib plus camrelizumab versus anlotinib plus S-1 as second-line therapy for advanced esophageal squamous cell carcinoma: A real-world retrospective study

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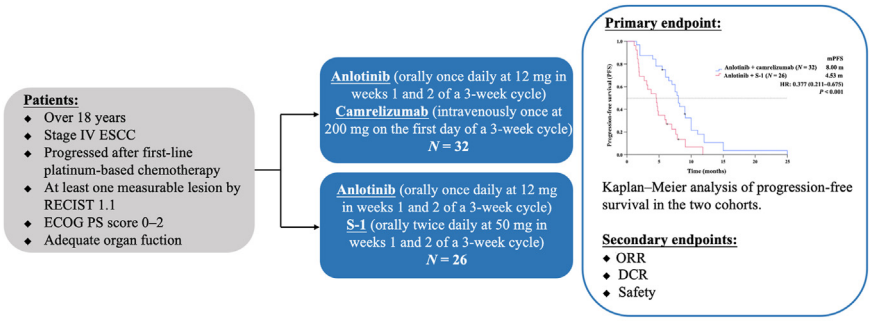
HIGHLIGHTS

- This retrospective study assessed the efficacy and safety of anlotinib plus camrelizumab as second-line therapy for advanced esophageal squamous cell carcinoma (ESCC).
- Anlotinib plus camrelizumab doublet regimen yielded a median progression-free survival (PFS) of 8.00 months in advanced ESCC.
- The combination of anlotinib plus camrelizumab shows promise as a second-line therapy for patients with advanced ESCC.

GRAPHICAL ABSTRACT



In this retrospective study, anlotinib plus camrelizumab doublet regimen as the second-line therapy showed better clinical activity than anlotinib plus S-1 doublet regimen in patients with advanced ESCC. DCR: Disease control rate; ECOG PS: Eastern Cooperative Oncology Group performance status; ESCC: Esophageal squamous cell carcinoma; HR: Hazard ratio; mPFS: Median progression-free survival; ORR: Objective response rate; PFS: Progression-free survival: RECIST 1.1: Response Evaluation Criteria in Solid Tumors version 1.1.

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ABSTRACT

Background: No data exist on the efficacy and safety of anlotinib plus camrelizumab doublet as second-line therapy for advanced esophageal squamous cell carcinoma (ESCC). Although anlotinib and the programmed death-1 (PD-1) inhibitor camrelizumab are used as treatments for ESCC, the combined use of anlotinib and camrelizumab as a second-line therapy has not been reported. Therefore, this study explored the efficacy and toxicity of anlotinib plus camrelizumab as second-line therapy for advanced ESCC.

Methods: Fifty-eight patients with advanced ESCC undergoing second-line therapy, either with anlotinib plus camrelizumab or anlotinib plus S-1, were enrolled and retrospectively analyzed at Jiangsu Province Hospital of Chinese Medicine from January 2020 to December 2021. The primary endpoint was progression-free survival (PFS), with secondary endpoints including the objective response rate (ORR), disease control rate (DCR), and assessment of toxicity.

Results: In patients with advanced ESCC, the anlotinib plus camrelizumab group ($N = 32$) exhibited longer PFS (8.00 *vs.* 4.53 months, $P < 0.001$), higher ORR (28.1 *vs.* 19.2%, $P = 0.431$), and higher DCR (87.5 *vs.* 65.4%,

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$P = 0.045$) than those in the anlotinib plus S-1 group ($N = 26$). Treatment-related adverse events (TRAEs) were predominantly grade 1/2 in both groups, with a higher incidence of grade 1/2 skin toxicity in patients treated with anlotinib plus camrelizumab ($P = 0.033$). Two patients (6.3%) developed grade 1/2 immune-related pneumonia. The incidence of grade 3/4 TRAEs did not differ significantly between the two groups. Multivariable Cox regression analysis identified that the drug regimen ($P < 0.001$), Eastern Cooperative Oncology Group performance status ($P = 0.008$), and differentiation grade ($P = 0.008$) were independent prognostic factors for PFS.

Conclusions: Anlotinib plus camrelizumab exhibited promising antitumor efficacy and manageable toxicity when used as a second-line treatment for advanced ESCC.

Introduction

Esophageal cancer (EC) is among the most prevalent cancers, ranking seventh in incidence (604,000 new cases) and sixth in mortality (544,000 deaths) in 2020.[1] More than half of EC cases occur in China, where the incidence and mortality rank sixth and fourth, respectively.[2] Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the main histological types, with ESCC predominant in China and EAC the leading type in Western countries.[2] Early detection of EC is challenging, often resulting in advanced or metastatic disease upon diagnosis. Therefore, patients with advanced or metastatic EC have a poor prognosis, with a 5-year survival rate below 30%.[3]

Platinum-based chemotherapy has been the standard first-line regimen for advanced ESCC in recent decades. First-line treatment with 5-fluorouracil plus cisplatin resulted in a median progression-free survival (mPFS) and overall survival (OS) of 4.80 and 10.40 months respectively.[4] However, the clinical efficacy of chemotherapy is limited. Programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) blockades interrupt immunosuppression via the PD-1/PD-L1 signaling pathway, restoring T cell function in the tumor microenvironment (TME).[5] Those blockades exhibit anti-tumor activity in various malignancies, including EC.[6] Recently, combining PD-1 blockades including pembrolizumab, nivolumab, camrelizumab, toripalimab, sintilimab, and tislelizumab with chemotherapy as first-line treatment for advanced ESCC have shown substantial improvements in PFS and OS compared with chemotherapy alone in trials including KEYNOTE-590, CheckMate 648, ESCORT-1st, JUPITER-06, ORIENT-15, and RATIONALE-306 respectively.[7–12] The ESCORT-1st trial, a phase 3 study on advanced or metastatic ESCC revealed that camrelizumab plus chemotherapy in the first-line treatment led to significantly longer mPFS (6.90 *vs.* 5.60 months, $P < 0.001$) and OS (15.30 *vs.* 12.00 months, $P = 0.001$) than the placebo plus chemotherapy group.[9] Thus, PD-1 blockades together with chemotherapy are recommended as the new standard first-line regimen in a series of key guidelines. In trials KEYNOTE-181, ATTRACTION-03, ESCORT, ORIENT-2, and RATIONALE-302, PD-1 blockade monotherapy as second or above-line treatment for patients with ESCC who failed first-line chemotherapy, exhibited better OS and reduced the risk of death.[13–17] In the phase 3 trial (ESCORT), camrelizumab monotherapy as a second-line treatment significantly prolonged median OS (mOS) (8.30 *vs.* 6.20 months, $P = 0.001$) and had a longer median duration of response (DOR) (7.40 *vs.* 3.40 months) than chemotherapy.[15] However, the benefit of patients with ESCC from second or further-line treatment remains limited owing to a lack of diverse treatment regimens.

Anlotinib is a novel oral small-molecule tyrosine kinase inhibitor (TKI) that targets the vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), c-Ret, and c-Kit.[18] In the ALTER-1102 trial, anlotinib treatment for patients with ESCC who experienced progression after first-line chemotherapy had a longer PFS (3.02 *vs.* 1.41 months) than with a placebo.[19] Based on this data, the 2019 Chinese Society of Clinical Oncology (CSCO) guideline recommended anlotinib monotherapy as a second or above-line treatment for ESCC.

Anlotinib can potentiate immune microenvironment normalization and exert synergistic therapeutic benefits when combined with PD-1 blockades.[20,21] Liu et al[22] investigated the efficacy of anlotinib plus PD-1 blockade as second or further-line therapy for advanced ESCC, revealing a 5.40-month mPFS longer than anlotinib monotherapy, with a mPFS of 3.02 months in the ALTER-1102 trial.[22] To date, the combination of anlotinib and camrelizumab as second-line therapy in ESCC has not been reported. Patients with advanced ESCC might benefit more from second-line combination therapy than later-line therapy. Therefore, this retrospective study aimed to investigate the efficacy and toxicity of anlotinib plus camrelizumab in patients with ESCC, specifically focusing on second-line therapy.

Methods

Patients and study design

This retrospective study systematically reviewed the electronic medical records of patients with EC receiving anlotinib plus camrelizumab or anlotinib plus S-1 as second-line therapy between January 2020 and December 2021 at the Jiangsu Province Hospital of Chinese Medicine. The inclusion criteria were as follows: (1) patients >18 years old; (2) ESCC confirmed by histopathology; (3) stage IV recurrent or metastatic ESCC as per tumor, node, metastasis (TNM) staging system; (4) at least one measurable lesion per Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1; (5) patients progressed after first-line paclitaxel (175 mg/m^2) plus cisplatin (75 mg/m^2) chemotherapy; (6) Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2; (7) life expectancy >3 months; (8) patients with adequate hematologic, hepatic, renal and cardiac functions. The key exclusion criteria were as follows: (1) other concurrent malignancies; (2) history of anti-angiogenic therapy; (3) active autoimmune diseases; (4) brain metastases; (5) uncontrolled hypertension after antihypertensive treatment; (6) severe heart, liver, and kidney dysfunction; (7) history of interstitial lung disease (ILD); (8) history of peptic ulcers, digestive tract perforation, or obstruction. Among the 85 patients with ESCC that were screened, 58 met the above criteria and were included in the study. Patients with advanced ESCC who received second-line treatment with anlotinib plus camrelizumab were grouped into cohort A ($N = 32$), and those receiving anlotinib plus S-1 were grouped into cohort B ($N = 26$).

Treatment schedule

Fifty-eight patients with ESCC in cohorts A and B were orally administered anlotinib (Chia Tai Tianqing Pharmaceutical, China) once daily at 12 mg in weeks 1 and 2 of a 3-week cycle. Meanwhile, 32 patients in cohort A were administered camrelizumab (Jiangsu Hengrui Pharmaceutical, China) once at 200 mg on the first day of the 3-week cycle. 26 patients in cohort B received S-1 (Shandong New Time Pharmaceutical, China) orally at 50 mg twice daily during weeks 1 and 2 of a 3-week cycle. The anlotinib dose was adjusted for patients who experienced grade 3 or higher serious adverse events (AEs) following symptomatic treatment. Patients received treatment until disease progression, unacceptable toxicity, or withdrawal of consent. Patients did not use other antitumor drugs, except for symptomatic treatment.

Efficacy evaluation

Computed tomography (CT) scans were performed at weeks 6 and 12 and subsequently, every 6–12 weeks until disease progression. The tumor response was assessed based on RECIST 1.1. Clinical efficacy included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The primary endpoint was PFS, defined as the time from treatment initiation to any recorded disease progression, death from any cause, or the last follow-up date. Patients lost to follow-up or not progressing at the time of analysis were censored at their last follow-up when PFS was examined. The secondary endpoints were objective response rate (ORR) and disease control rate (DCR). The ORR was defined as the percentage of patients who achieved CR or PR. The DCR was defined as the percentage of patients who achieved CR, PR, or SD.

Treatment-related adverse events assessment

Routine blood, urine, stool, liver, kidney function, cardiac function, coagulation function, electrocardiogram, and CT were evaluated before treatment initiation. Except for the CT scan, these tests were scheduled for every treatment cycle. Toxicity data were collected during treatment. Treatment-related AEs (TRAEs) were graded from 1 to 5 according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (CTCAE 5.0).

Statistical analyses

By using Power Analysis and Sample Size Software 2023 (PASS 2023) (NCSS, LLC, Kaysville, Utah, USA), 28 PFS events would provide 90% power for a long-rank test, assuming a hazard ratio (HR) of 0.556 for anlotinib plus camrelizumab relative to anlotinib (corresponding to a mPFS of 5.40 months *vs.* 3.02 months), with a two-sided α of 0.05.[23]

PFS was estimated using the Kaplan–Meier method and compared between groups using the log-rank test. HRs and the associated 95% confidence intervals (CIs) were calculated using a Cox proportional hazards model. The ORR and DCR were compared between the groups using the Pearson's chi-squared test or Fisher's exact test, as appropriate. Univariate and multivariate Cox regression analyses were performed to investigate the influence of multiple factors on PFS. Statistical Package for the Social Sciences (SPSS) software (version 26.0; IBM Corp.) was used for statistical analysis. All statistical tests were two-sided, and the results were considered statistically significant at $P < 0.05$. Follow-up data were collected via outpatient and inpatient electronic medical records or via phone. The follow-up deadline was December 31, 2022.

Results

Clinical characteristics

Between January 2020 and December 2021, 58 eligible patients with ESCC were enrolled. Among them, 32 received anlotinib plus camrelizumab (cohort A), and 26 received anlotinib plus S-1 (cohort B). Forty-eight (82.8%) patients were males and 10 (17.2%) were females. The median age was 65 years (52–80 years) in cohort A and 68 years (55–86 years) in cohort B. The two groups had similar clinical characteristics, including sex, age, ECOG PS score, tumor location, differentiation grade, metastasis, radical surgery history, and radiotherapy history with $P > 0.05$. The clinical characteristics are detailed in Table 1.

Table 1
Baseline characteristics of 58 patients with ESCC.

Characteristics	Anlotinib plus camrelizumab ($N = 32$), n (%)	Anlotinib plus S-1 ($N = 26$), n (%)	χ^2	P value
Sex			1.074	0.487
Male	25 (78.1)	23 (88.5)		
Female	7 (21.9)	3 (11.5)		
Age			1.240	0.266
≤65 years	17 (53.1)	10 (38.5)		
>65 years	15 (46.9)	16 (61.5)		
ECOG PS score			0.052	0.820
0–1	20 (62.5)	17 (65.4)		
2	12 (37.5)	9 (34.6)		
Tumor location			0.012	0.994
Upper	7 (21.9)	6 (23.1)		
Middle	15 (46.9)	12 (46.2)		
Lower	10 (31.2)	8 (30.7)		
Differentiation grade			0.003	0.956
Low	17 (53.1)	14 (53.8)		
Medium–high	15 (46.9)	12 (46.2)		
Metastases			0.000	0.983
Lymph nodes	25 (78.1)	17 (65.4)		
Distal organs	16 (50.0)	11 (42.3)		
Prior radical surgery			0.510	0.475
Yes	19 (59.4)	13 (50.0)		
No	13 (40.6)	13 (50.0)		
Prior radiotherapy			2.698	0.100
Yes	11 (34.4)	4 (15.4)		
No	21 (65.6)	22 (84.6)		
PD-L1 CPS score				
<1%	6 (18.8)	/	/	/
≥1%	7 (21.9)	/		
Not detected	19 (59.3)	/		

The PD-L1 CPS score was defined as the number of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) as a proportion of the total number of tumor cells multiplied by 100. CPS: Combined positive score; ECOG PS: Eastern Cooperative Oncology Group performance status; ESCC: Esophageal squamous cell carcinoma; PD-L1: Programmed death-ligand 1.

Efficacy and survival

As of the December 31, 2022 data cutoff, the median follow-up time was 25.75 months (10.77–34.90 months) in cohort A and 30.22 months (9.00–41.47 months) in cohort B. Follow-up loss occurred in four patients (6.9%), two (6.3%) from cohort A and two (7.7%) from cohort B [Figure 1]. At the follow-up deadline, 26 patients (81.3%) in cohort A and 24 (92.3%) in cohort B had PD. The mPFS was significantly longer in patients who received anlotinib plus camrelizumab than in those who received anlotinib plus S-1 (8.00 *vs.* 4.53 months, $P < 0.001$) [Figure 2]. At the data cutoff, eight deaths (25%) were recorded in Cohort A and seven (26.9%) in Cohort B. The mOS of the two cohorts was not reached. Fifty-eight patients with ESCC were evaluated for clinical efficacy, including 32 treated with anlotinib plus camrelizumab and 26 treated with anlotinib plus S-1. No CR was observed among the 58 patients in cohorts A and B. Nine patients (28.1%) achieved PR in cohort A, whereas five (19.2%) achieved PR in cohort B. Cohort A consisted of 19 patients (59.4%) evaluated for SD and 12 (46.2%) in cohort B. The ORRs were 28.1 and 19.2% in cohorts A and B, respectively ($P = 0.431$). The DCR in cohort A (87.5%) was significantly higher than that in cohort B (65.4%) ($P = 0.045$). Details of the clinical responses of the 58 patients are summarized in Table 2. The waterfall plots [Figure 3A and B] show the percent change in the sum of the longest diameter of measurable lesions from the baseline for each patient.

Toxicity

In this retrospective study, TRAEs in the two cohorts were primarily grade 1 or 2. The predominant grade 1/2 TRAEs included fatigue (37.5% in cohort A, 38.5% in cohort B, $P = 0.094$), hypertension (31.1% in cohort A, 26.9% in cohort B, $P = 0.719$), hypothyroidism (28.1% in cohort A, 19.2% in cohort B, $P = 0.431$), myelosuppression (25.0% in cohort A, 23.1% in cohort B, $P = 0.865$), hand-foot syndrome (31.1% in cohort A, 30.8% in cohort B, $P = 0.969$), skin toxicity (25.0% in cohort A, 3.8% in cohort B, $P = 0.033$), nausea and vomiting (21.9% in cohort A, 11.5% in cohort B, $P = 0.487$), diarrhea (15.6% in cohort A, 7.7% in cohort B, $P = 0.442$), proteinuria (21.9% in cohort A, 15.4% in cohort B, $P = 0.738$), and liver dysfunction (12.5% in cohort A, 11.5% in cohort B,

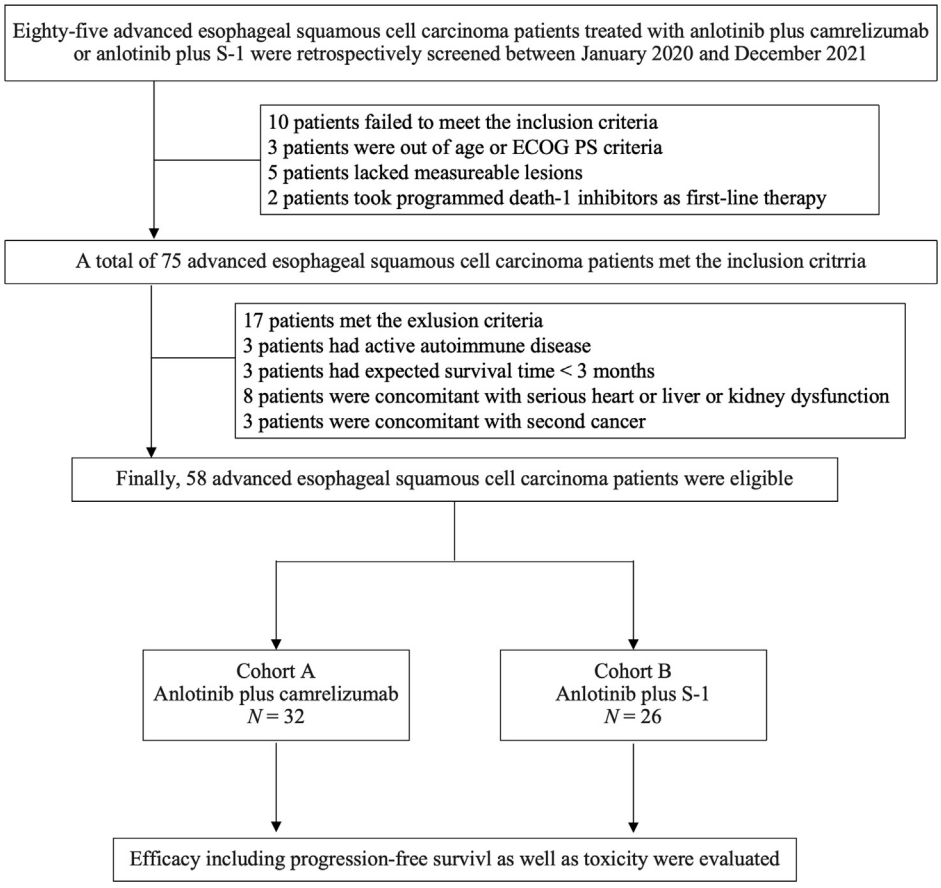

Figure 1. Flow chart of the retrospective, single-arm, real-world study. ECOG PS: Eastern Cooperative Oncology Group performance status.

P = 1.000). Two patients (6.3%) in cohort A developed grade 1/2 immune-related interstitial pneumonia, whereas none did in cohort B. Grade 3/4 TRAEs in cohorts A and B. In cohort A, there was one patient who developed grade 3/4 hypertension, hand-foot syndrome, and liver dysfunction. Only one patient developed grade 3/4 hypertension. None of the patients discontinued treatment because of intolerable TRAEs. All TRAEs were clinically manageable. No treatment-related death occurred during the study period. The TRAEs are listed in Table 3.

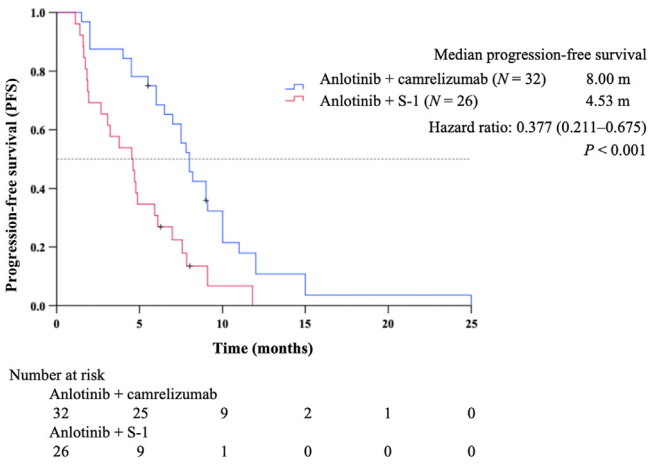

Figure 2. Kaplan–Meier plot depicts the PFS of patients in both cohorts. The anlotinib plus camrelizumab cohort showed a longer mPFS than the anlotinib plus S-1 cohort (P < 0.001). mPFS: Median progression-free survival; PFS: Progression-free survival.

Univariate analysis

Univariate analysis revealed that sex, age, tumor location, prior radical surgery, prior radiotherapy, lymph node metastasis, and distal organ metastasis had no influence on mPFS (P > 0.05). However, the ECOG PS, differentiation grade, and drug regimen were reliable prognostic factors. In addition, an ECOG PS ≤ 1 (P = 0.026) and a medium–high differentiation grade (P = 0.035) were identified as positive factors. The results are summarized in Table 4.

Multivariate Cox regression analysis

Significant variables (P < 0.05) in the univariate analysis were subsequently included in a multivariate Cox regression model. The results indicated that the drug regimen, ECOG PS, and differentiation grade were independent factors affecting PFS in patients with advanced ESCC, as shown in Table 5. As shown in Figure 4A and B, the mPFS of patients with an ECOG PS of 0–1 was 7.50 months, and that of patients with an ECOG PS of 2 was 3.77 months (P = 0.002). Patients with a medium-to-high differentiation grade had a significantly higher mPFS than patients with a low differentiation grade (7.83 *vs.* 4.50 months, P < 0.001). As shown in the forest plot [Figure 5], a longer PFS was achieved with anlotinib plus camrelizumab than with anlotinib plus S-1 in all tested subgroups.

Programmed death-ligand 1 status and age affecting progression-free survival

PD-L1 expression was assessed in 13 patients from the anlotinib plus camrelizumab group. Seven patients with a PD-L1 combination positive score (CPS) ≥1% had a significantly longer mPFS (10.00 *vs.* 8.00 months, P = 0.037) than the six patients with a CPS <1% [Table 6].

Table 2
Efficacy overview for 58 patients with advanced ESCC treated with either anlotinib plus camrelizumab or anlotinib plus S-1.

Efficacy	Anlotinib plus camrelizumab ($N = 32$), n (%)	Anlotinib plus S-1 ($N = 26$), n (%)	χ^2	P value
Complete response	0 (0)	0 (0)	/	/
Partial response	9 (28.1)	5 (19.2)	/	/
Stable disease	19 (59.4)	12 (46.2)	/	/
Progressive disease	4 (12.5)	9 (34.6)	/	/
Objective response rate	9 (28.1)	5 (19.2)	0.620	0.431
Disease control rate	28 (87.5)	17 (65.4)	4.034	0.045*

* indicates statistical significance at $P < 0.05$. Responses were evaluated according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). ESCC: Esophageal squamous cell carcinoma; RECIST 1.1: Response Evaluation Criteria in Solid Tumors version 1.1.

A longer mPFS (9.10 *vs.* 7.50 months, $P = 0.474$) was achieved in patients >75 years in the anlotinib plus camrelizumab group [Figure 6A]. In contrast, in the anlotinib plus S-1 group, patients ≤75 years had a significantly longer mPFS (4.60 *vs.* 1.63 months, $P = 0.029$) than those >75 years [Figure 6B]. In both cohorts, patients >75 years showed a shorter mPFS (4.70 *vs.* 6.50 months, $P = 0.815$) [Figure 6C].

Discussion

Till now, there is no standard second-line regimen for patients with advanced ESCC. The majority of second-line treatments had short PFS. The clinical demand for high PFS second-line therapy is far from satisfied. Our data revealed that anlotinib plus camrelizumab significantly prolonged the mPFS by 3.47 months (8.00 *vs.* 4.53 months, $P = 0.001$) with an HR of 0.377 in patients with advanced ESCC after first-line chemotherapy. Additionally, the TRAEs profile of anlotinib plus camrelizumab was tolerable and clinically manageable. Thus, our results demonstrated that anti-angiogenic TKIs combined with PD-1 blockade may be a superior treatment regimen for patients with ESCC who have received first-line therapy.

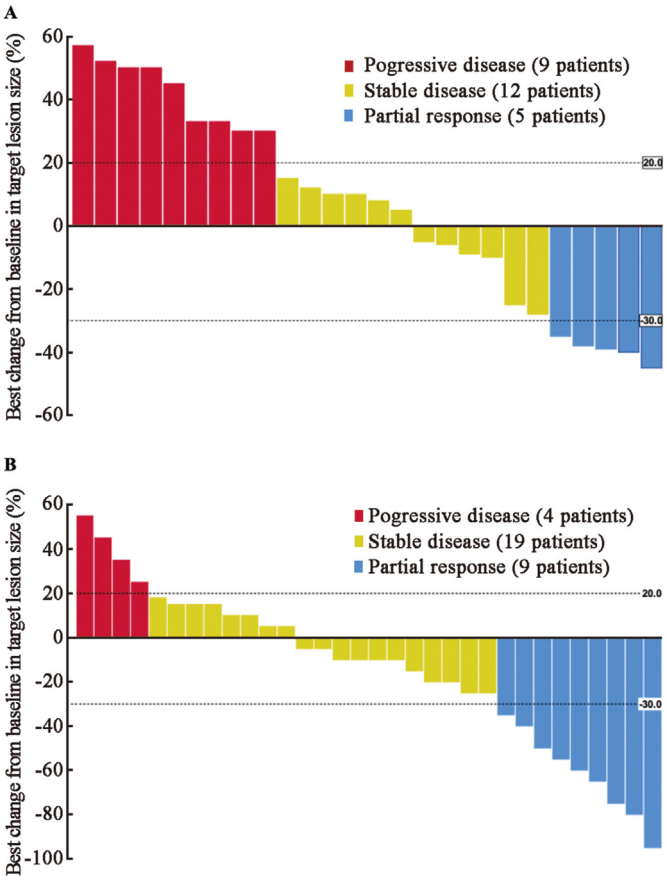

Figure 3. Waterfall plot illustrating the maximum change in target lesion size based on the tumor response in patients treated with anlotinib plus S-1 or anlotinib plus camrelizumab. (A) Anlotinib plus S-1. (B) anlotinib plus camrelizumab.

In a randomized, double-blind, multicenter phase 2 trial (ALTER-1102), Huang et al[19] investigated the efficacy of anlotinib monotherapy as a second- or further-line treatment for patients with previously treated recurrent or metastatic ESCC. The ALTER-1102 data revealed longer mPFS (3.02 *vs.* 1.41 months, $P < 0.001$) in the anlotinib monotherapy group than that in the placebo group. The ORR and DCR for the anlotinib monotherapy were 7.3 and 64% respectively. In our study, the anlotinib plus S-1 group showed higher mPFS (4.53 *vs.* 3.02 months) and ORR (19.2 *vs.* 7.3%) than those in the ALTER-1102 study. However, the DCR (65.4 *vs.* 64%) in both studies were similar. The prolonged mPFS of the anlotinib plus S-1 group in this study may be attributed to the higher ORR than DCR. In the ESWN 01 study, S-1 monotherapy in patients previously treated for recurrent or metastatic ESCC showed an mPFS of 1.70 months and an ORR of 9.7%.[24] Comparatively, the anlotinib plus S-1 in our study showed consistent mPFS and ORR compared to the combination of anlotinib in ALTER-1102 and S-1 in the ESWN 01. The results suggested that combining anti-angiogenic TKIs with the fluoropyrimidine derivative S-1 may be a viable option for patients after first-line platinum or taxane-based chemotherapy failure.

In this retrospective study, patients treated with anlotinib plus camrelizumab achieved a longer mPFS (8.00 *vs.* 4.53 months, $P = 0.001$) and higher DCR (87.5 *vs.* 65.4%, $P = 0.045$) than those treated with anlotinib plus S-1. In the ESCORT phase 3 study, camrelizumab monotherapy as a second-line treatment for patients with advanced ESCC yielded an mPFS of 1.90 months (95% CI: 1.90–2.40 months) and a mOS of 8.30 months (95% CI: 6.80–9.70 months), with a 29% reduced risk of death compared to chemotherapy[15] As shown in clinical trials KEYNOTE-181, ATTRACTION-03, ORIENT-2 and RATIONALE-302,[13,14,16,17] the mPFS for PD-1 blockades in second-line treatment for ESCC did not exceed 1.90 months. The combination of anlotinib and camrelizumab resulted in a much longer mPFS (8.00 *vs.* 1.90 months) than camrelizumab monotherapy as second-line therapy for ESCC, possibly attributed to immune microenvironment normalization by anlotinib and synergistic therapeutic benefits of the two agents.[20,21]

PD-L1 expression is considered a predictor of immunotherapy response in various cancers, including non-small cell lung cancer (NSCLC).[25] In KEYNOTE-181, patients with PD-L1 CPS ≥10% had a longer OS compared with chemotherapy for ESCC.[13] In the Checkmate-577 subgroup analysis, patients with a PD-L1 tumor positive score (TPS) ≥ 5% benefit more after surgery.[26] In this study, among the 13 patients in the anlotinib plus camrelizumab group, seven patients with a PD-L1 CPS ≥1% had a significantly longer mPFS (10.00 *vs.* 8.00 months, $P = 0.037$) than the six patients with CPS <1%. This data aligns with the results from the ESCORT trial, which

Table 3
Treatment-related adverse events in both cohorts.

Adverse events	Grades 1 and 2, *n* (%)			Grades 3 and 4, *n* (%)		
	Cohort A (*N* = 32)	Cohort B (*N* = 26)	*P* value	Cohort A (*N* = 32)	Cohort B (*N* = 26)	*P* value
Fatigue	12 (37.5)	10 (38.5)	0.940	0 (0)	0 (0)	0
Hypertension	10 (31.3)	7 (26.9)	0.719	1 (3.1)	1 (3.8)	1.000
Hypothyroidism	9 (28.1)	5 (19.2)	0.431	0 (0)	0 (0)	0
Myelosuppression	8 (25.0)	6 (23.1)	0.865	0 (0)	0 (0)	0
Hand-foot syndrome	10 (31.3)	8 (30.8)	0.969	1 (3.1)	0 (0)	1.000
Skin toxicity	8 (25.0)	1 (3.8)	0.033*	0 (0)	0 (0)	0
Nausea and vomiting	7 (21.9)	3 (11.5)	0.487	0 (0)	0 (0)	0
Diarrhea	5 (15.6)	2 (7.7)	0.442	0 (0)	0 (0)	0
Proteinuria	7 (21.9)	4 (15.4)	0.738	0 (0)	0 (0)	0
Liver dysfunction	4 (12.5)	3 (11.5)	1.000	1 (3.1)	0 (0)	1.000
Interstitial pneumonia	2 (6.3)	0 (0)	1.000	0 (0)	0 (0)	0

* indicates statistical significance at $P < 0.05$.

showed that the OS benefit of camrelizumab over chemotherapy in the second-line therapy of ESCC is independent of PD-L1 expression.[15] However, tumor mutation burden (TMB), mismatched repair protein deficiency (dMMR), microsatellite instability-high (MSI-H), and tumor immune microenvironment (TIME) classification model are also considered biomarkers for predicting treatment efficacy in ESCC.[27–29] Thus, accurate biomarkers for predicting optimal clinical benefits from immunotherapy require further investigation.

Almost all studies, including those on anlotinib or camrelizumab, only enrolled patients ≤75 years. However, our study included 12

Table 4
Univariate analysis of PFS of 58 patients with advanced ESCC.

Characteristics	Patients, *n*	Median progression-free survival (months), 95% *CI*	Hazard ratio, 95% *CI*	*P* value
Sex			1.673 (0.816–3.429)	0.160
Male	48	6.000 (4.235–7.765)		
Female	10	7.830 (5.614–10.046)		
Age			1.047 (0.606–1.809)	0.869
≤65 years	27	6.500 (4.726–8.274)		
>65 years	31	6.100 (3.019–9.181)		
ECOG PS score			0.527 (0.300–0.926)	0.026*
0–1	37	7.500 (6.009–8.991)		
2	21	3.770 (0.779–6.761)		
Tumor location			/	0.596
Upper	13	5.500 (3.959–7.041)		
Middle	27	6.970 (2.720–11.220)		
Lower	18	6.500 (4.629–8.371)		
Differentiation grade			1.796 (1.042–3.094)	0.035*
Low	31	4.500 (2.914–6.086)		
Medium–high	27	7.830 (7.236–8.424)		
Lymph node metastasis			1.438 (0.761–2.717)	0.263
Yes	42	6.100 (4.813–7.387)		
No	16	4.770 (0.000–10.591)		
Distal organ metastasis			0.747 (0.429–1.299)	0.301
Yes	27	6.100 (1.537–10.663)		
No	31	6.500 (5.144–7.856)		
Prior radical surgery			0.635 (0.363–1.114)	0.113
Yes	32	6.500 (4.726–8.274)		
No	26	5.500 (2.928–8.072)		
Prior radiotherapy			1.219 (0.657–2.262)	0.529
Yes	15	6.000 (2.483–9.517)		
No	43	6.500 (5.123–7.877)		
Treatment regimen			0.377 (0.211–0.675)	<0.001*
Anlotinib + camrelizumab	32	8.000 (7.059–8.941)		
Anlotinib + S-1	26	4.530 (2.694–6.366)		

* indicates statistical significance at $P < 0.05$. CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status; ESCC: Esophageal squamous cell carcinoma.

Table 5
Multivariate Cox regression analysis of PFS of 58 patients with advanced ESCC.

Clinical characteristics	Hazard ratio	95% *CI*	*P* value
ECOG PS score (0–1 *vs.* 2)	0.378	0.205–0.698	0.002*
Differentiation grade (low *vs.* medium–high)	2.559	1.435–4.564	<0.001*
Regimen (anlotinib + camrelizumab *vs.* anlotinib + S-1)	0.250	0.133–0.471	<0.001*

* indicates statistical significance at $P < 0.05$. CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status; ESCC: Esophageal squamous cell carcinoma; PFS: Progression-free survival.

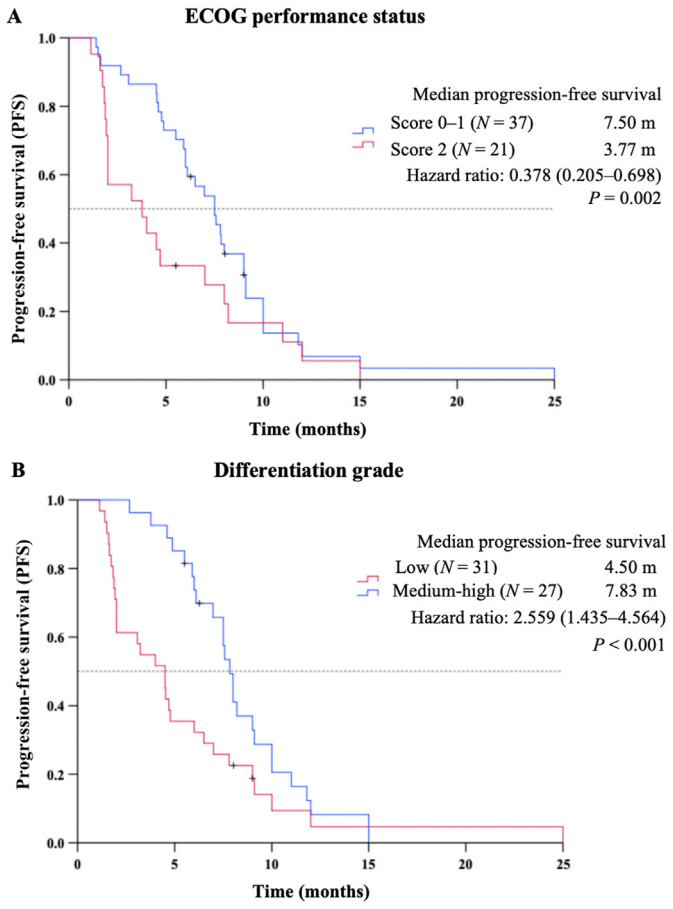

Figure 4. Kaplan–Meier plot curves for the PFS of patients with different ECOG PS scores and differentiation grades. (A) Patients with an ECOG PS score of 0–1 had a longer mPFS (7.50 *vs.* 3.77 months, $P = 0.008$) than those with an ECOG PS score of 2. (B) Patients with a medium–high differentiation grade showed higher mPFS than those with a low differentiation grade ($P = 0.008$). ECOG PS: Eastern Cooperative Oncology Group performance status; mPFS: Median progression-free survival; PFS: Progression-free survival.

patients (20.7%) >75 years old. In the anlotinib plus S-1 group, patients ≤75 years old had a significantly longer mPFS (4.60 *vs.* 1.63 months) than those >75 years. Alternatively, patients >75 years old in the anlotinib plus camrelizumab group had a longer mPFS (9.10 *vs.* 7.50 months).

Table 6
Survival analysis of patients based on PD-L1 status.

Factor	Patients, *n*	Median progression-free survival (months)	95% *CI*	*P* value
PD-L1 status				0.037*
<1%	6	8.000	3.559–12.441	
≥1%	7	10.000	7.026–12.974	

* indicates statistical significance at $P < 0.05$. CI: Confidence interval; PD-L1: Programmed death ligand 1.

Cuffe et al[30] elucidated that elderly patients with NSCLC had a survival benefit from adjuvant chemotherapy, whereas the benefit in patients ≥80 years old remains uncertain and warrants further investigation. An international cohort study showed that single-agent immune checkpoint inhibitors are effective and generally well tolerated in older patients, even >85 years with, cancer.[31] Considering our findings, patients >75 years old may benefit from a combination of moderate anti-angiogenic TKI and PD-1 blockade treatment.

Compared to the study by Liu et al,[22] which also investigated the efficacy of anlotinib plus PD-1 blockade, this study presented a longer mPFS (8.00 *vs.* 5.40 months) and higher ORR (28.1 *vs.* 23.9%) and DCR (87.5 *vs.* 71.7%). This could be attributed to several factors. In the study by Liu et al, 20 patients (41.7%) were administered anlotinib plus a PD-1 blockade after second-line treatment, whereas our study focused on patients who received second-line therapy. In addition, the study by Liu et al[22] included only 19 patients (39.6%) who received anlotinib plus camrelizumab treatment, with the remainder receiving anlotinib plus toripalimab (11 patients), sintilimab (12 patients), and pembrolizumab (six patients). This complexity of regimen combinations may confound efficacy.

However, this study had several limitations. This was a single-arm, retrospective, real-world study with a relatively small sample size. Therefore, future randomized, multicenter, phase 3 trials may provide more robust evidence for second-line anlotinib plus camrelizumab in treating ESCC. Second, OS was not determined owing to the short follow-up period. Lastly, PD-L1 expression data did not include all patients, potentially compromising the relationship between PD-L1 expression and immunotherapy response.

In conclusion, anlotinib plus camrelizumab as second-line therapy showed promising survival outcomes among patients with advanced ESCC. The toxicity profiles were primarily grade 1/2, tolerable, and manageable. The combination of anlotinib and camrelizumab could emerge as a new therapeutic option for patients with advanced ESCC after first-line chemotherapy failure.

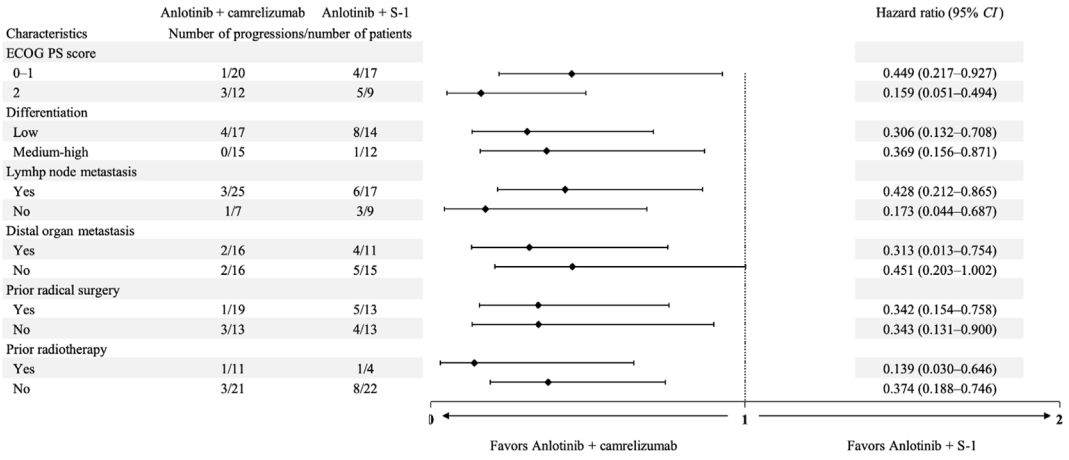

Figure 5. Forest plot for subgroup analysis of PFS. Hazard ratios and the corresponding 95% CIs for anlotinib plus camrelizumab or anlotinib plus S-1 were calculated using the Cox proportional hazards model. CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status; PFS: Progression-free survival.

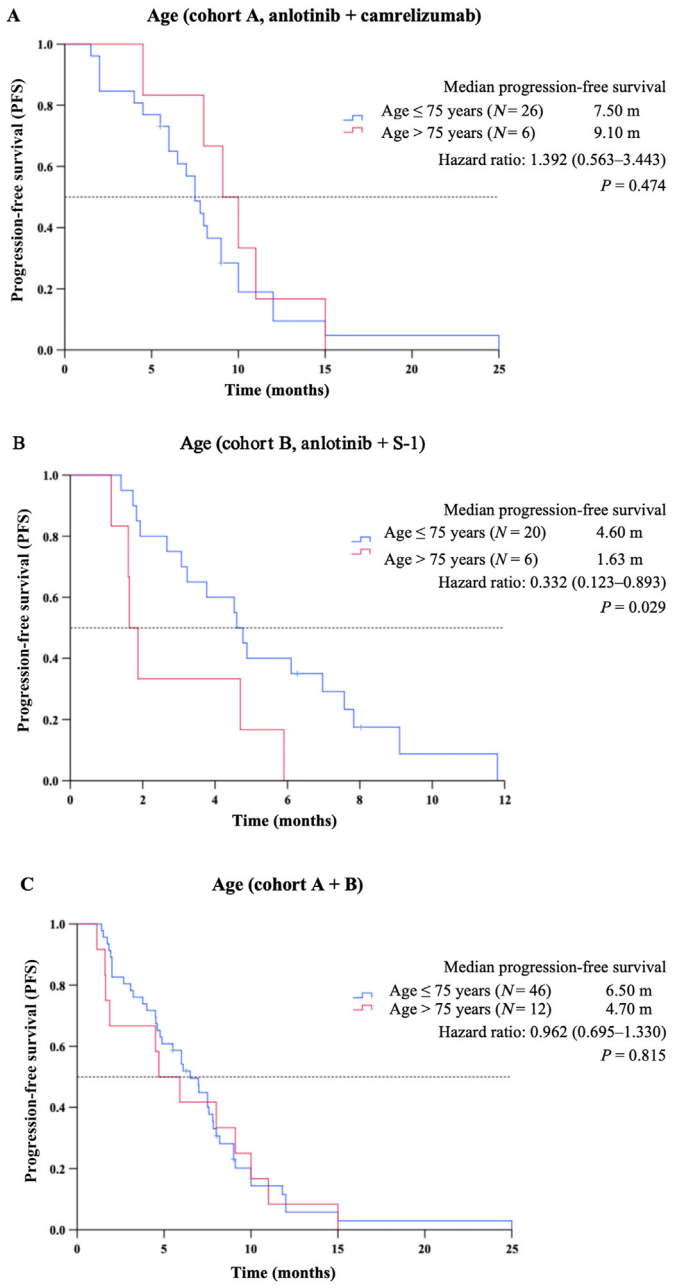

Figure 6. Kaplan–Meier plot for the PFS of patients at older age (>75 years). (A) In cohort A, patients >75 years had a longer mPFS. (B) In cohort B, patients >75 years had a significantly shorter mPFS. (C) In both cohorts, patients >75 years showed a shortened mPFS. mPFS: Median progression-free survival; PFS: Progression-free survival.

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Authors contribution

Wei Zhang: conceptualization, data curation, methodology, formal analysis, data review, and interpretation; Mingyu Chen: conceptualization, data curation, methodology, formal analysis, data review, and interpretation; Hong Dai: data review and interpretation; Wei Sun: conceptualization, data curation, methodology, formal analysis, data review and interpretation, funding acquisition, and supervision. All authors have written and reviewed the manuscript.

Ethics statement

This retrospective study involving human participants was conducted in accordance with the principles of the *Declaration of Helsinki* and its later amendments. The study was approved by the local ethics committee of the Jiangsu Province Hospital of Chinese Medicine (No. 2020NL-088-07). Written informed consent was obtained upon the last follow-up.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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