Am J Cancer Res 2025;15(3):1321-1334
www.ajcr.us /ISSN:2156-6976/ajcr0161158

Original Article

Outcomes and influencing factors of targeted combination immunotherapy in advanced esophageal cancer patients following immunochemotherapy failure

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Received October 14, 2024; Accepted February 28, 2025; Epub March 15, 2025; Published March 30, 2025

Abstract: Background: Advanced esophageal cancer presents significant treatment challenges, especially after immunochemotherapy failure. This study evaluates the efficacy of further treatment with combination chemotherapy versus combination immunotherapy crossover in terms of tumor regression, quality of life, and identifies factors influencing treatment outcomes. Methods: In a retrospective case-control study, clinical data from 293 patients with advanced esophageal cancer treated at Shanxi Province Cancer Hospital between February 2021 and February 2023 were analyzed. Patients excluded from radical resection due to immunochemotherapy failure were divided into two groups: 95 received combination chemotherapy with Irinotecan and Tigio (S-1, Tegafur/Gimeracil/Oteracil Potassium), and 198 underwent Anlotinib combined with immunotherapy crossover. Treatment efficacy was assessed using tumor regression grading (TRG), and quality of life was evaluated using EORTC QLQ-C30 and QLQ-OES18 scales. Potential factors affecting treatment efficacy were examined using multivariate logistic regression analysis. Results: Baseline characteristics, including age, gender, body mass index (BMI), and history of smoking and alcohol consumption, were comparable between the two groups. TRG showed no significant differences in distribution, with objective response rates of 40% in the Irinotecan/S-1 group and 44.44% in the combined immunotherapy crossover group (P = 0.472). However, quality of life measures indicated superior outcomes from immunotherapy crossover in physical (P = 0.024), emotional (P = 0.002), and general health scores (P = 0.003). Factors negatively impacting treatment success included male gender, smoking, alcohol consumption history, and certain tumor locations. Elevated CEA levels positively correlated with treatment efficacy. Logistic regression analysis identified male gender (OR, 2.109; P = 0.021), smoking (OR, 2.575; P = 0.003), alcohol consumption (OR, 1.995; P = 0.043), and CEA levels (OR, 0.742; P = 0.017) as significant predictors of treatment efficacy. Conclusion: Both immunotherapy and combination chemotherapy showed comparable efficacy in tumor regression. However, combination chemotherapy improved certain aspects of quality of life. Factors such as gender, lifestyle habits, and CEA levels can significantly influence treatment outcomes.

Keywords: Influencing factors, targeted, immunotherapy, advanced esophageal cancer patients, immunochemotherapy failure

Introduction

Esophageal cancer remains a major oncological challenge, ranking as the sixth leading cause of cancer-related mortality worldwide [1]. Despite advances in diagnostic and therapeutic strategies, the prognosis for patients with advanced disease remains poor, with a 5-year survival rate of less than 20% [2]. Current treatment paradigms often involve a combination of chemotherapy, radiotherapy, and, where feasible, surgical resection, aimed at achieving locoregional control and symptom palliation [3]. The emergence of immunotherapy, particularly

immune checkpoint inhibitors, has opened new therapeutic avenues by modulating the immune system to target cancer cells [4].

Over the past decade, immune checkpoint inhibitors, such as programmed death-1 (PD-1) blockers and PD-ligand 1 (PD-L1) inhibitors, have revolutionized the treatment of various solid tumors, including esophageal cancer [5, 6]. Despite their initial success, many patients experience primary or acquired resistance, highlighting the need for more effective therapeutic strategies [7]. Combination therapies, which integrate immunotherapy with other modalities like chemotherapy or targeted agents, are emerging as viable strategies to overcome resistance and potentiate antitumor efficacy [8, 9]. However, the identification of patients who most likely benefit from such combinations remains challenging, emphasizing the need to understand the influencing factors governing treatment responses.

Several studies have suggested that the efficacy of immunotherapy and its combinations are influenced by a myriad of patient-specific and tumor-related factors, including genomic alterations, tumor microenvironment characteristics, and patient demographics [10]. Understanding the impact of these variables is crucial for optimizing immunotherapy regimens and identifying patients who are most likely to benefit. In esophageal cancer specifically, the role of these factors in modulating responses to combination immunotherapy following the failure of initial chemotherapeutic regimens remains poorly elucidated, presenting a critical knowledge gap.

The histological diversity of esophageal cancer, primarily comprising squamous cell carcinoma and adenocarcinoma, further complicates the treatment landscape [11]. Each histological subtype has distinct genetic and molecular characteristics, potentially affecting their respective responses to immunotherapy. Squamous cell carcinoma, prevalent in Eastern countries, and adenocarcinoma, more common in Western populations, may diverge in their interaction with the immune system and response to checkpoint inhibitors [12]. Therefore, investigating the influence of tumor histology and other patient factors is crucial for personalizing treatment and improving clinical outcomes.

Beyond tumor biology, demographic factors such as age, sex, and lifestyle choices like smoking and alcohol consumption are believed to modulate immune function and, consequently, the efficacy of immunotherapeutic interventions. Hormonal differences between males and females might contribute to variations in immune responses, while lifestyle factors can alter immune system dynamics, further influencing treatment outcomes [13]. Therefore, treatment decisions should consider these factors to tailor therapies and maximize clinical benefit across diverse patient populations.

Given these considerations, this study aims to evaluate the outcomes and influencing factors of targeted combination immunotherapy in patients with advanced esophageal cancer who have failed prior immunochemotherapy.

Materials and methods

Case selection

This retrospective case-control study included 293 patients with advanced esophageal cancer treated at Shanxi Province Cancer Hospital from February 2021 to February 2023. These patients were all non-responders to immunochemotherapy at their initial treatment and were divided into two groups based on their subsequent treatment approach: the Irinotecan/S-1 group (n = 95) and the combined immunotherapy crossover group (n = 198).

Patient demographic information, such as general characteristics, tumor regression grading, and quality of life, was collected from medical records. The study aimed to analyze tumor biological characteristics and immune function indicators in relation to various treatment outcomes. The study was approved by the Institutional Review Board and Ethics Committee of Shanxi Province Cancer Hospital. Informed consent was waived due to the retrospective nature of the study and the use of de-identified patient data, which ensured no risk or impact on patient care.

Inclusion and exclusion criteria

Inclusion criteria: 1) Histologically confirmed esophageal cancer, classified as stage III to IV according to the 8th edition of the American

Joint Committee on Cancer; 2) Previous treatment with combination immunotherapy and chemotherapy, with patients deemed unsuitable for radical resection; 3) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 [14]; 4) Age between 18 and 70 years; 5) Adequate organ function; 6) Expected survival of at least three months; 7) Complete medical records with no missing data.

Exclusion criteria: 1) Presence of a tracheoesophageal fistula, active infection, interstitial pneumonia, severe cardiovascular disease, malignant pleural or pericardial effusion, or any other concurrent cancer; 2) Immune system deficiency or autoimmune disease; 3) Presence of other tumors.

Treatment methods

The Irinotecan/S-1 group: On the first day, an intravenous infusion of irinotecan (180 mg/m^2, 90 min) was administered. Tigio (S-1, Tegafur/Gimeracil/Oteracil Potassium) capsules were administered based on body surface area, with dosages as follows: 60 mg for > 1.5 m^2, 50 mg for 1.25-1.5 m^2, and 40 mg for ≤ 1.25 m^2, twice a day. The medication was administered after breakfast and dinner for 1-14 days, with 21 days as one cycle.

The combined immunotherapy crossover group: Immune checkpoint inhibitors (ICIs) such as Camrelizumab, Sintilimab, or Pembrolizumab (200 mg dose) were administered on day 1 of each cycle, with a treatment cycle of 21 days. The original immunotherapy drug was continued in conjunction with Anlotinib treatment, 10 mg orally once daily for 14 days, followed by a 7-day rest period. Treatment continued until disease progression, which was defined by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [15]. Disease progression was identified by an increase of more than 20% in the sum of the longest diameters of target lesions compared to the last evaluation, with an absolute increase of at least 5 millimeters, or the appearance of new lesions.

Three months after treatment, small tissue samples were collected via endoscopic biopsy for pathological examination. Pathological examination results were evaluated based on TRG and classified into three levels according to criterion [16]: TRG0 (pathological complete response, pCR): No visible viable cancer cells. TRG1 (near-complete response): Single cells or sparse small groups of cancer cells. TRG2 (partial response): Residual lesions showing evident tumor regression, but beyond single cells or sparse small groups. TRG3 (poor or no response): A large number of residual cancer cells with no evident tumor regression. Based on the pathological assessments and therapeutic efficacy, patients received combined immunotherapy crossover treatment were further categorized into an effective group (n = 88, TRG0 and TRG1) and an ineffective group (n = 110, TRG2 and TRG3) to identify risk factors influencing treatment efficacy.

Quality of life assessment for esophageal cancer patients

Patient recovery was assessed using the six-minute walk test, following standard operational guidelines [17]. Three months after treatment, during follow-up visits, the quality of life was evaluated using the QLQ-C30 and QLQ-OES18 scales. The QLQ-C30 comprises 30 items covering five functional domains (physical, role, emotional, social, and cognitive functioning), three symptom domains (fatigue, pain, and nausea/vomiting), and one general health status domain, along with six single items related to quality of life. The QLQ-OES18 is a specialized subscale of the QLQ-C30 specific to esophageal cancer. Together, these scales assess the quality of life for patients with esophageal cancer.

The EORTC QLQ-OES18 includes 18 items focusing on symptoms and side effects related to esophageal cancer, divided into four domains (dysphagia, eating, reflux, and pain) and six symptom items (trouble with saliva, choking, dry mouth, taste, cough, and speech). Scores from the QLQ-C30 and QLQ-OES18 were converted into a 0-100 scale using a linear transformation. On the functional scale, higher scores reflect better function, while higher scores in general health imply a better quality of life. Conversely, higher scores on the symptom scales or single items indicate more severe symptoms. The Cronbach's α coefficient for the QLQ-C30 scale was 0.927, indicating high reliability [18].

Immune function indicators

Prior to treatment, 5 mL of peripheral venous blood was collected from patients in both groups and placed in a heparin anticoagulant tube for 20 minutes. The samples were centrifuged at 3,000 rpm with an 8 cm radius for 10 minutes. The supernatant was then extracted and stored at -20°C for subsequent testing. A flow cytometer (Merck Life Sciences, Merck Chemical Technology Co., Ltd.) was employed to measure the levels of Natural Killer (NK) cells, cluster of differentiation 4 positive T cells ($CD4^+$), cluster of differentiation 8 positive T cells ($CD8^+$), and the $CD4^+/CD8^+$ ratio.

Tumor biomarker levels

Prior to treatment, 5 mL of fasting venous blood was collected from patients in both groups. The samples were allowed to stand at room temperature for 30 minutes, followed by centrifugation at 3,000 rpm with an 8 cm radius for 10 minutes. The supernatant was then extracted and stored at -20°C for future analysis. Enzyme-linked immunosorbent assay (ELISA) was employed to measure the levels of cytokeratin 19 fragment (Cyfra21-1), squamous cell carcinoma antigen (SCC Ag), and serum carcinoembryonic antigen (CEA).

Statistical analyses

The minimum sample size was calculated using G*Power with a significance level (α) of 0.05 and a power (1-β) of 0.95. The minimum required sample size was determined to be 88. The sample size calculation was performed using the following formula: $n = [(Z_1 - \alpha/2 + Z_1 - \beta)/d]^2 \times [p_1(1 - p_1) + p_2(1 - p_2)]$.

Where: $Z_{1-\alpha}/2$ is the standard normal deviate corresponding to the desired significance level (1.96 for $\alpha = 0.05$). $Z_{1-\beta}$ is the standard normal deviate corresponding to the desired power (1.645 for power = 0.95). d is the effect size (the difference in proportions between two groups). p1 and p2 are the expected proportions in the two groups.

Statistical analysis was performed using SPSS version 29.0 (SPSS Inc., Chicago, IL, USA). Categorical data were represented as [n (%)]. The chi-square test was applied, with results expressed as χ^2. Continuous variables were first assessed for normality using the Shapiro-Wilk test. Normally distributed data were analyzed using t-tests and presented as mean ± standard deviation ($\bar{x} \pm s$). A *p*-value of less than 0.05 was considered statistically significant.

Correlation analysis for continuous variables was performed using Pearson's correlation, while Spearman's correlation was used for categorical variables. Initially, correlation analysis was conducted to evaluate relationships between variables. Subsequently, multiple logistic regression analysis was performed to determine the independent effect of each variable on the study outcome, accounting for potential confounding factors.

Results

Impact of different treatment approaches on patients with advanced esophageal cancer who failed immunochemotherapy

Baseline characteristics: The mean age was similar in both groups, with 60.36 ± 2.18 years in the irinotecan/S-1 group and 60.27 ± 2.34 years in the combined immunotherapy crossover group (*P* = 0.764) (**Table 1**). Gender distribution was comparable, with males constituting 58.95% and 60.19% of the participants in the irinotecan/S-1 and combined immunotherapy crossover groups, respectively (*P* = 0.851). The two groups had similar BMI, with means of 22.14 ± 3.61 in the irinotecan/S-1 group and 22.32 ± 3.77 in the combined immunotherapy crossover group (*P* = 0.691). Smoking (53.68% vs. 54.55%, *P* = 0.890) and alcohol consumption (65.26% vs. 68.18%, *P* = 0.618) histories did not differ significantly between the two groups. Employment status, educational degree, marital status, hypertension, diabetes prevalence, and tumor location were also comparable between groups (all *P* > 0.05). Consequently, baseline characteristics were well balanced between the two treatment groups.

Pathological examination: The treatment outcomes between the two groups were assessed based on TRG and objective response rates (**Table 2**). The distribution of TRG showed no significant difference between the two groups, with TRG0 observed in 21 and 51 patients, TRG1 in 17 and 37 patients, TRG2 in 53 and 101 patients, and TRG3 in 4 and 9 patients in

Table 1. Baseline characteristics of participants

Parameters	Irinotecan/S-1 group (n = 95)	Combined immunotherapy crossover group (n = 198)	t/χ^2	P
Age (years)	60.36 ± 2.18	60.27 ± 2.34	0.301	0.764
Gender (Male/Female)	56 (58.95%)/39 (41.05%)	119 (60.10%)/79 (39.90%)	0.036	0.851
Body Mass Index (kg/m^2)	22.14 ± 3.61	22.32 ± 3.77	0.398	0.691
Smoking history [n (%)] (Y/N)	51 (53.68%)/44 (46.32%)	108 (54.55%)/90 (45.45%)	0.019	0.890
Drinking history [n (%)] (Y/N)	62 (65.26%)/33 (34.74%)	135 (68.18%)/63 (31.82%)	0.248	0.618
Employment [n (%)] (Y/N)	41 (43.16%)/54 (56.84%)	83 (41.92%)/115 (58.08%)	0.040	0.841
Degree of education (0: Junior high school and below/1: high school/2: college diploma or above)			1.329	0.514
Junior high school and below	44 (46.32%)	96 (48.48%)		
High school	31 (32.63%)	71 (35.86%)		
College diploma or above	20 (21.05%)	31 (15.66%)		
Marital Status [n/(%)] (0: Married/1: Single/2: Divorced)			0.643	0.725
Married	78 (82.11%)	169 (85.35%)		
Single	3 (3.16%)	4 (2.02%)		
Divorced	14 (14.74%)	25 (12.63%)		
Hypertension [n (%)] (Y/N)	50 (52.63%)/45 (47.37%)	94 (47.47%)/104 (52.53%)	0.683	0.409
Diabetes [n (%)] (Y/N)	47 (49.47%)/48 (50.53%)	96 (48.48%)/102 (51.52%)	0.025	0.874
Tumor location [n (%)] (0: Proximal esophagus/1: Middle esophagus/2: Distal esophagus/3: Esophagogastric junctiona)			0.227	0.973
Proximal esophagus	9 (9.47%)	21 (10.61%)		
Middle esophagus	14 (14.74%)	31 (15.66%)		
Distal esophagus	45 (47.37%)	94 (47.47%)		
Esophagogastric junctiona	27 (28.42%)	52 (26.26%)		

Table 2. Comparison of treatment outcome between two groups of patients

Parameters	Irinotecan/S-1 group (n = 95)	Combined immunotherapy crossover group (n = 198)	χ^2	P
TRG0	21	51		
TRG1	17	37		
TRG2	53	101		
TRG3	4	9		
Objective response rate [n (%)]	38 (40%)	88 (44.44%)	0.517	0.472

Note: TRG = tumor regression grade.

Table 3. Comparison of scores of quality of life (QLQ-C30) of patients in two groups after treatment

Parameters		Irinotecan/S-1 group (n = 95)	Combined immunotherapy crossover group (n = 198)	t	P
Functional domain	Physical function	72.22 ± 12.98	75.9 ± 12.94	2.276	0.024
	Role function	88.27 ± 3.48	88.99 ± 3.65	1.601	0.111
	Emotional function	50.84 ± 3.47	52.87 ± 7.85	3.065	0.002
	Social Functions	86.13 ± 5.32	87.01 ± 6.52	1.228	0.221
	Cognitive function	80.36 ± 11.17	79.69 ± 16.54	0.412	0.681
General health condition		58.21 ± 4.56	58.85 ± 4.36	2.963	0.003

the irinotecan/S-1 group and the combined immunotherapy crossover group, respectively. The objective response rate was 40% (38 of 95) in the irinotecan/S-1 group compared to 44.44% (88 of 198) in the combined immunotherapy crossover group ($P = 0.472$), indicating similar efficacy between the two treatment modalities.

Quality of life rating: Physical function scores were significantly higher in the combined immunotherapy crossover group (75.90 ± 12.94) compared to the irinotecan/S-1 group (72.22 ± 12.98; $P = 0.024$) (**Table 3**). Emotional function also improved significantly in the combined immunotherapy crossover group, with scores of 52.87 ± 7.85 versus 50.84 ± 3.47 in the irinotecan/S-1 group ($P = 0.002$). General health condition scores similarly favored the combined immunotherapy crossover group (59.85 ± 4.36 vs. 58.21 ± 4.65; $P = 0.003$). Role function, social function, and cognitive function scores showed no significant differences between the groups (all $P > 0.05$). These results suggest that combination immunotherapy may enhance certain aspects of quality of life in advanced esophageal cancer patients post-immunochemotherapy failure.

Dysphagia scores were similar between the two groups (38.25 ± 11.31 vs. 38.57 ± 11.25; $P = 0.819$), as were scores for eating difficulties (31.63 ± 7.36 vs. 31.06 ± 7.22; $P = 0.531$) and reflux symptoms (41.02 ± 4.76 vs. 41.78 ± 7.11; $P = 0.278$) (**Table 4**). Pain levels were virtually identical in both groups (21.35 ± 4.39 vs. 21.65 ± 7.24; $P = 0.662$). Symptom scores for saliva swallowing, choking, dry mouth, decreased appetite, cough, and speaking showed no significant differences between groups (all $P > 0.05$). These findings indicate that the addition of combination immunotherapy with crossover did not significantly change esophagus-specific quality of life symptoms compared to combined chemotherapy in patients following immunochemotherapy failure.

Factors influencing the efficacy of targeted combination immunotherapy following immunochemotherapy failure in patients with advanced esophageal cancer

Baseline characteristics: Gender distribution showed a significantly higher proportion of males in the ineffective group (69.09%) compared to the effective group (47.73%; $P = 0.002$). Smoking history was also more prevalent in the ineffective group (63.64% vs. 42.05%; $P = 0.002$). Additionally, a higher percentage of patients in the ineffective group had a history of alcohol consumption compared to the effective group (75.45% vs. 55.68%; $P =$

Table 4. Comparison of scores of quality of life (QLQ-OES18) of patients in two groups after treatment

Parameters		Irinotecan/S-1 group (n = 95)	Combined immunotherapy crossover group (n = 198)	t	P
Domain	Dysphagia	38.25 ± 11.31	38.57 ± 11.25	0.229	0.819
	Eating	31.63 ± 7.36	31.06 ± 7.22	0.628	0.531
	Reflux	41.02 ± 4.76	41.78 ± 7.11	1.086	0.278
	Pain	21.35 ± 4.39	21.65 ± 7.24	0.437	0.662
Symptom	Saliva swallowing	23.25 ± 0.34	23.34 ± 3.11	0.405	0.686
	Choking	42.52 ± 3.06	42.91 ± 7.63	0.620	0.536
	Dry mouth	13.56 ± 7.39	13.48 ± 5.97	0.092	0.927
	Decreased Appetite	14.37 ± 6.94	14.28 ± 6.37	0.110	0.912
	Cough	22.96 ± 4.36	22.95 ± 4.15	0.020	0.984
	Speaking	24.37 ± 0.91	24.47 ± 0.68	0.951	0.343

0.003). Employment status approached significance, with a higher proportion of employed individuals in the effective group than in the invalid group (50.00% vs. 36.36%; $P = 0.054$). Other characteristics, including age, BMI, education level, marital status, hypertension, and diabetes, showed no significant differences between the groups (all $P > 0.05$). The detailed results are shown in **Table 5**. These findings suggest that gender, smoking, and alcohol consumption history may be influencing factors for treatment efficacy among advanced esophageal cancer patients.

Tumor characteristics: The analysis of tumor biological characteristics before treatment revealed a significant difference in tumor location distribution between the ineffective group and effective group ($P = 0.024$) (**Table 6**). More patients in the effective group had tumors located at the distal esophagus compared to the invalid group (53.41% vs. 42.73%), whereas tumors in the proximal and middle esophagus were more frequent in the ineffective group (12.73% and 20.91%, respectively) than in the effective group (6.82% and 7.95%, respectively). Tumor length did not significantly differ between the ineffective group and the effective group (4.19 ± 1.02 cm vs. 4.36 ± 1.27 cm; $P = 0.299$). These results suggest that while tumor location may influence treatment outcomes, tumor length were consistent across groups.

Immune function: The percentage of $CD4^+$ T cells was similar between the ineffective group (26.79 ± 3.58) and the effective group (27.05 ± 1.24; $P = 0.482$). Likewise, the percentage of $CD8^+$ T cells showed no significant difference between the two groups (30.77 ± 1.25 vs. 30.94 ± 0.66, $P = 0.210$). The $CD4^+/CD8^+$ ratio was slightly higher in the ineffective group compared to the effective group (1.21 ± 0.11 vs. 1.17 ± 0.22), but the difference was not statistically significant ($P = 0.134$). Additionally, NK cell percentages were comparable between groups, with 22.14 ± 2.38 in the invalid group and 21.92 ± 2.44 in the effective group ($P = 0.509$). See **Table 7** for details. These findings indicate that there were no significant differences in baseline immune function indicators between the groups, suggesting other factors may be more critical for the treatment efficacy.

Tumor markers: As shown in **Figure 1**, the level of CEA was significantly higher in the effective group (10.79 ± 1.34) compared to the ineffective group (10.21 ± 1.27; $P = 0.002$). Similarly, Cyfra21-1 levels were higher in the effective group (9.02 ± 0.87) than in the ineffective group (8.77 ± 0.74; $P = 0.031$). Conversely, levels of SCC Ag were comparable between the groups (11.38 ± 3.22 vs. 10.94 ± 3.69; $P = 0.372$). These results suggest that elevated CEA and Cyfra21-1 levels may be associated with a more effective treatment response in advanced esophageal cancer patients following immunochemotherapy failure.

Correlation analysis: Gender showed a negative correlation with treatment efficacy (rho = -0.216, $P = 0.002$), indicating that male gender may be associated with less effective outcomes. Both smoking and alcohol consumption histories were also negatively correlated with treatment efficacy (rho = -0.215, $P = 0.002$ and rho = -0.208, $P = 0.003$, respectively), suggest-

Table 5. Baseline characteristics of participants

Parameters	Invalid group (n = 110)	Effective group (n = 88)	t/χ^2	p
Age (years)	60.77 ± 3.24	60.15 ± 3.71	1.248	0.213
Gender (Male/Female)	76 (69.09%)/34 (30.91%)	42 (47.73%)/46 (52.27%)	9.267	0.002
Body Mass Index (kg/m^2)	22.26 ± 2.17	22.09 ± 2.31	0.546	0.586
Smoking history [n (%)] (Y/N)	70 (63.64%)/40 (36.36%)	37 (42.05%)/51 (57.95%)	9.176	0.002
Drinking history [n (%)] (Y/N)	83 (75.45%)/27 (24.55%)	49 (55.68%)/39 (44.32%)	8.601	0.003
Employment [n (%)] (Y/N)	40 (36.36%)/70 (63.64%)	44 (50.00%)/44 (50.00%)	3.722	0.054
Degree of education (0: Junior high school and below/1: high school/2: college diploma or above)			2.221	0.329
Junior high school and below	55 (50.00%)	39 (44.32%)		
High school	39 (35.45%)	29 (32.95%)		
College diploma or above	16 (14.55%)	20 (22.73%)		
Marital Status [n/(%)] (0: Married/1: Single/2: Divorced)			5.905	0.052
Married	86 (78.18%)	80 (90.91%)		
Single	4 (3.64%)	1 (1.14%)		
Divorced	20 (18.18%)	7 (7.95%)		
Hypertension [n (%)] (Y/N)	52 (47.27%)/58 (52.73%)	47 (53.41%)/41 (46.59%)	0.736	0.391
Diabetes [n (%)] (Y/N)	49 (44.55%)/61 (55.45%)	48 (54.55%)/40 (45.45%)	1.956	0.162

Table 6. Tumor biological characteristics of two groups of patients before treatment

Parameters	Invalid group (n = 110)	Effective group (n = 88)	t/χ^2	P
Tumor location [n (%)] (0: Proximal esophagus/1: Middle esophagus/2: Distal esophagus/3: Esophagogastric junction)			9.480	0.024
Proximal esophagus	14 (12.73%)	6 (6.82%)		
Middle esophagus	23 (20.91%)	7 (7.95%)		
Distal esophagus	47 (42.73%)	47 (53.41%)		
Esophagogastric junction	26 (23.64%)	28 (31.82%)		
Tumor length (cm)	4.19 ± 1.02	4.36 ± 1.27	1.043	0.299

Table 7. Comparison of immune function indicators between two groups of patients before treatment

Parameters	Invalid group (n = 110)	Effective group (n = 88)	t	P
$CD4^+$/%	26.79 ± 3.58	27.05 ± 1.24	0.705	0.482
$CD8^+$/%	30.77 ± 1.25	30.94 ± 0.66	1.258	0.210
$CD4^+/CD8^+$	1.21 ± 0.11	1.17 ± 0.22	1.509	0.134
NK cells/%	22.14 ± 2.38	21.92 ± 2.44	0.662	0.509

Note: $CD4^+$ = Cluster of Differentiation 4 Positive T Cells; $CD8^+$ = Cluster of Differentiation 8 Positive T Cells; NK cells = Natural Killer cells.

ing these factors may adversely affect treatment success. Conversely, tumor location demonstrated a positive correlation with efficacy (rho = 0.179, *P* = 0.011), implying that the specific site of the tumor could influence therapeutic response favorably. In terms of tumor markers, CEA levels showed a positive correlation with efficacy (rho = 0.207, *P* = 0.003), as did Cyfra21-1, although to a lesser extent (rho = 0.149, *P* = 0.036). The details are shown in **Table 8**. These findings suggest that gender, smoking and alcohol consumption history, tumor location, and specific tumor marker levels are influential factors for treatment response in this patient group.

Multivariate logistic regression analysis: A multivariate logistic regression analysis (**Table 9**) was conducted to identify factors influencing the therapeutic efficacy of targeted combination therapy. The analysis revealed that several factors were statistically significant. Gender was found to be a significant predictor, with a coefficient of 0.746 (P = 0.021), indicating that male patients had a higher likelihood of treatment failure, with an odds ratio (OR) of 2.109 (95% CI: 1.117-3.984). Similarly, a history of smoking was significantly associated with an increased risk of suboptimal outcome, with a coefficient of 0.946 (P = 0.003) and an OR of 2.575 (95% CI: 1.375-4.821). Alcohol consumption history also influenced the outcomes, with a coefficient of 0.691 (P = 0.043) and an OR of 1.995 (95% CI: 1.023-3.888). In contrast, tumor location had a negative coefficient of -0.357 with borderline significance (P = 0.051), suggesting a trend towards lower risk of failure in certain tumor locations, although this finding was not statistically significant at the traditional 0.05 level, with an OR of 0.700 (95% CI: 0.489-1.002). Additionally, carcinoembryonic antigen (CEA) levels were inversely related to treatment failure (coefficient -0.299, P = 0.017), with decreased failure likelihood associated with higher CEA, indicating an OR of 0.742 (95% CI: 0.580-0.949). Although the Cyfra21-1 marker also showed a negative coefficient of -0.369 (P = 0.070), suggesting a possible predictive value, it did not reach statistical significance, with an OR of 0.691 (95% CI: 0.463-1.031). Therefore, these findings highlight gender, smoking and alcohol consumption history, and CEA levels as significant factors contributing to the outcomes of targeted combination therapy in patients who have experienced immunochemotherapy failure.

Discussion

In this study, we investigated the outcomes and factors influencing the efficacy of targeted combination therapy in patients with advanced esophageal cancer who experienced failure following initial immunochemotherapy. Our results suggest that various demographic and treatment-related variables modulate the efficacy of subsequent therapeutic interventions, highlighting the complexity and heterogeneity of treatment responses in advanced esophageal cancer.

A key finding was the observed impact of gender on treatment outcomes, where male patients demonstrated a lower treatment efficacy compared to their female counterparts. This sex-based disparity can potentially be attributed to biological differences in immune system function and male hormonal profiles, which influence immune modulation. Previous studies have shown that estrogen may enhance immune surveillance and anti-tumor responses, whereas androgens could have an immunosuppressive effect [19]. These hormonal differences might contribute to altered immune landscape and differential therapeutic responses observed between genders.

In line with this, we observed negative correlations of smoking and alcohol consumption histories with treatment efficacy. Smoking and alcohol consumption are well-known risk fac-

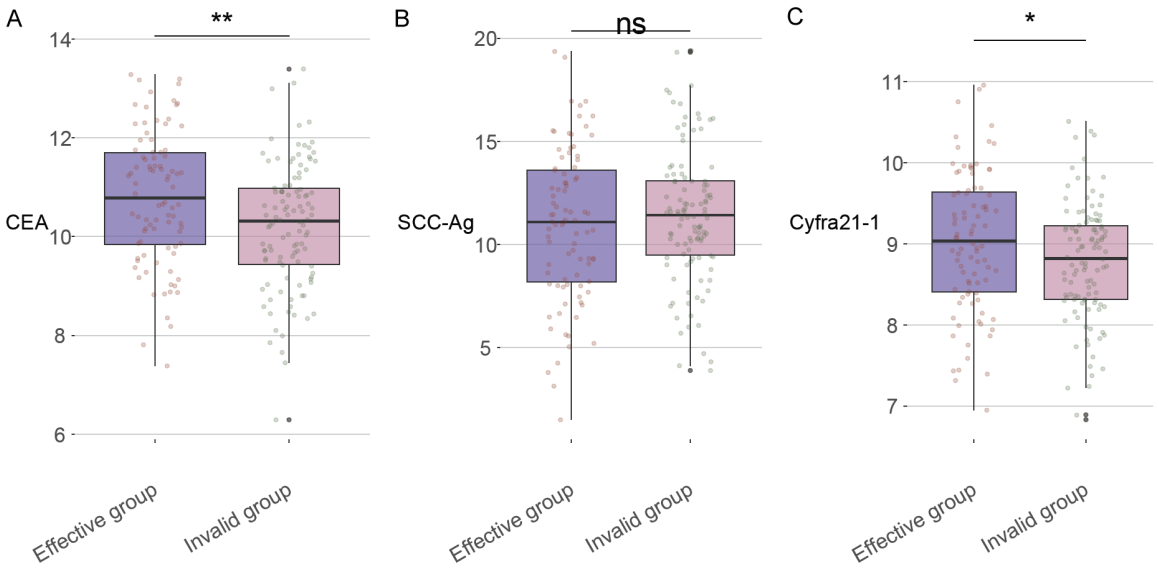

Figure 1. Comparison of tumor marker levels between two groups of patients before treatment. A: CEA; B: SCC-Ag; C: Cyfra21-1. Note: CEA = Carbohydrate Antigen; SCC-Ag = Squamous Cell Carcinoma Antigen; Cyfra21-1 = Cytokeratin 19 fragment.

Table 8. Correlation analysis of various factors with the efficacy of targeted combination immunotherapy

Parameters	rho	*P* value
Gender (Male/Female)	-0.216	0.002
Smoking history (Y/N)	-0.215	0.002
Drinking history (Y/N)	-0.208	0.003
Tumor location (0: Proximal esophagus/1: Middle esophagus/2: Distal esophagus/3: Esophagogastric junctiona)	0.179	0.011
CEA	0.207	0.003
Cyfra21-1	0.149	0.036

Table 9. Multivariate logistic regression analysis of influencing factors and invalid outcome of targeted combination immunotherapy

	Coefficient	Std. Error	Wald Stat	P	OR	OR CI Lower	OR CI Upper
Gender	0.746	0.324	2.301	2.109	1.117	3.984	0.021
Smoking history	0.946	0.320	2.956	2.575	1.375	4.821	0.003
Drinking history	0.691	0.341	2.028	1.995	1.023	3.888	0.043
Tumor location	-0.357	0.183	-1.950	0.700	0.489	1.002	0.051
CEA	-0.299	0.126	-2.377	0.742	0.580	0.949	0.017
Cyfra21-1	-0.369	0.204	-1.809	0.691	0.463	1.031	0.070

tors that not only contribute to the etiology of esophageal cancer but also alter systemic immune responses. Smoking has been associated with impaired neutrophil and lymphocyte function, as well as with increased levels of systemic inflammation and oxidative stress [20, 21], which can undermine the treatment efficacy by promoting an immunosuppressive tumor microenvironment. Similarly, alcohol consumption can alter cytokine profiles and reduce the activation and proliferation of immune effector cells, thereby dampening the response to immunotherapeutic interventions. Furthermore, these behaviors are often more prevalent in male patients, who also showed a lower efficacy of treatment in our study. This suggests that gender, in conjunction with smoking and drinking, may play a significant role in

the overall treatment response. The higher prevalence of these risk factors among males, combined with potential biological differences such as hormonal influences, could contribute to the observed disparities in treatment outcomes. Future studies should explore the specific mechanisms through which these factors interact and influence treatment efficacy, potentially leading to more tailored therapeutic strategies.

The role tumor location plays in influencing treatment outcomes, particularly with distal esophageal tumors showing a more favorable response, raises questions about tumor biology and the local microenvironment's implications [22]. It is hypothesized that the heterogeneity in vascular supply and lymphatic drainage across different esophageal segments might account for this variance [23]. Distal esophageal cancers might exhibit distinct biomolecular profiles or be more accessible to both drug delivery and immune infiltration due to anatomical differences [24]. Understanding these localized differences is vital, as they could offer clues towards optimizing treatment modalities and developing new tumor-specific strategies.

Tumor biomarker analysis introduced intriguing insights. The biomarkers in our study have been widely used in the clinical setting for esophageal cancer to monitor disease progression and treatment response. Specifically, CEA and Cyfra21-1 have diagnostic and prognostic value. In our study, elevated pre-treatment levels of CEA and Cyfra21-1 were associated with improved outcomes. However, may not be consistent with previous studies, which have often reported that elevated levels of these biomarkers are associated with diagnosis and recurrence monitoring [25, 26]. The discrepancy could be attributed to several factors, including the heterogeneity of patient populations, differences in treatment regimens, and variations in the timing of biomarker measurement. For instance, the dynamic changes in CEA and Cyfra21-1 levels during the course of treatment might reflect different biological processes, such as the activation of immune responses or the release of tumor antigens, which could influence the therapeutic response. While traditionally high levels of these markers indicate advanced disease burden, their response to treatment might reflect underlying tumor regression better in patients who remain most responsive to therapy [27]. The correlation may suggest that patients with higher levels are initially more susceptible to therapeutic intervention or that these markers play a role in immunogenic cell death or affect immune modulation positively during treatment intervention. The exact mechanisms by which biomarkers correlate with enhanced response require further molecular investigation, which might reveal potential pathways or adaptative immune responses that are more vigilant during combination therapy.

In examining immune function indicators, the lack of a significant difference in baseline T-cell or NK cell populations between effective and ineffective groups initially suggests limited diagnostic utility. However, it underlines an opportunity for dynamic monitoring of these markers post-treatment initiation. Changes over time in these populations, rather than baseline levels, might provide a richer picture of individual therapeutic response and adjustment needs.

While our study identified several significant factors associated with treatment efficacy, it is essential to consider the complex biological mechanisms underlying these associations. For instance, the interaction between immune checkpoint inhibitors and specific cellular pathways might be influenced by pre-existing conditions such as smoking or drinking habits and biological variables such as sex or tumor characteristics. These interactions could modulate the immune milieu within the tumor microenvironment, affecting the infiltration and activation of immune effector cells [28, 29]. Moreover, the genetic and epigenetic heterogeneity inherent to esophageal tumors could alter susceptibility to therapies, with certain genetic mutations conferring resistance or sensitivity to specific treatments [30].

The use of combination therapy approaches, as explored in this study, offers a potential strategy to overcome resistance mechanisms associated with monotherapies. By integrating immunotherapies with traditional chemotherapeutic agents or targeted inhibitors, such strategies aim to exploit synergistic effects that enhance overall therapeutic efficacy. However, the identification of factors such as those highlighted here is crucial for optimizing such combinations and personalizing treatment regimens to the

individual patient, thus ensuring the highest likelihood of success. Furthermore, these results underscore the importance of leveraging biomarkers and patient demographics in predicting and improving treatment outcomes. By understanding the factors that influence therapeutic efficacy, clinicians can better stratify and identify patients who are likely to benefit from specific treatment modalities, while sparing others from ineffective interventions and potential adverse effects. This could lead to a more personalized approach to esophageal cancer treatment, aligning therapeutic strategies with individual patient profiles.

This study's limitations warrant acknowledgment as they provide context to the findings and suggest directions for future research. First, the relatively small sample size may limit the generalizability of the results, necessitating larger, multicenter trials to validate these findings. The retrospective nature of the study introduces potential biases in data collection and analysis, which could affect the reliability of conclusions drawn. The study's reliance on de-identified data might also obscure patient-specific nuances that are crucial for understanding individual responses to therapy. Additionally, the investigation primarily focused on a limited set of biomarkers and immune function indicators, potentially overlooking other critical factors that could influence treatment outcomes. Addressing these limitations in future research could enhance the robustness and applicability of therapeutic interventions for advanced esophageal cancer. Finally, the effectiveness of the subsequent combination immunotherapy may be influenced by the type of original immunotherapy received. Different immune checkpoint inhibitors (ICIs) have distinct mechanisms of action, and their prior use could shape the tumor microenvironment and immune cell landscape, potentially affecting the response to the follow-up regimen. For example, the presence of pre-existing anti-tumor T-cell responses primed by the initial ICI treatment might enhance the efficacy of the combined therapy. Conversely, the development of resistance mechanisms or the exhaustion of effector T cells due to prolonged exposure to ICIs could diminish the benefits of additional treatments. Future studies should investigate the specific impact of each ICI on the outcomes of subsequent combination therapies to tailor more effective treatment strategies for patients with advanced esophageal cancer.

Conclusion

In conclusion, this study confirms that the combination of immunotherapy and targeted therapy can enhance immune sensitivity by altering the tumor microenvironment. Providing important scientific basis for future immunotherapy cross-line treatment. Rather than nullifying established therapies, these insights provide the groundwork for refining treatment protocols, tailoring them to patient profiles more comprehensively. Through continued research in this direction, a surprisingly nuanced picture of cancer treatment efficacy will emerge, one that is more in tune with the individualistic nature of cancer and responsive to the myriad factors influencing successful outcomes.

Disclosure of conflict of interest

None.

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