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Original Article

Re-challenge with immune checkpoint inhibitors as second-line treatment in advanced esophageal squamous cell carcinoma: a real-world multicenter retrospective study

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Abstract: Objectives: To evaluate the feasibility and efficacy of immune checkpoint inhibitor (ICI) rechallenge as second-line therapy in advanced esophageal squamous cell carcinoma (ESCC) patients who had progressed after first-line ICI-based treatment. Methods: This retrospective multicenter study analyzed 171 advanced ESCC patients who progressed after first-line ICI-based therapy and were subsequently re-treated with ICIs between 2021 and 2024. Rechallenge was defined as re-administration of the same or a different ICI agent. Patients were stratified by first-line treatment duration (≥180 vs. <180 days). Primary outcomes included progression-free survival during second-line treatment (PFS2) and overall survival (OS). Secondary outcomes included objective response rate (ORR), disease control rate (DCR), durable clinical benefit (DCB), and immune-related adverse events (irAEs). Results: Patients who had a first-line treatment duration of ≥180 days had significantly longer PFS2 (5.70 vs. 3.47 months, P<0.001) and OS (14.77 vs. 12.92 months, P = 0.008). Among second-line strategies, immunotherapy alone provided the longest PFS2, while ICI plus chemotherapy resulted in the shortest (P<0.001). PD-L1 expression ≥1% was paradoxically associated with shorter PFS2. IrAEs during second-line treatment were not associated with improved efficacy. Conclusions: ICI rechallenge is a feasible and effective option for selected ESCC patients, particularly those with ≥180 days of benefit from first-line ICI therapy. Immunotherapy alone or combined with antiangiogenic agents may be preferable over combination with chemotherapy. Further prospective studies are needed to identify predictive factors and optimize rechallenge strategies.

Keywords: Esophageal squamous cell carcinoma, immune checkpoint inhibitors, ICI rechallenge, second-line therapy, real-world study, progression-free survival, immune-related adverse events

Introduction

In 2022, China reported approximately 187,500 deaths and 224,000 new cases of esophageal carcinoma (EC), with esophageal squamous cell carcinoma (ESCC) accounting for roughly 85% of these cases [1]. The 5-year survival rate for metastatic, recurrent, or persistent ESCC is between 10 and 20% [2]. Immune checkpoint inhibitors (ICIs) combined with chemotherapy represent the frontline treatment for advanced ESCC, demonstrating prolonged progression-free survival (PFS) and overall survival (OS) in several large-scale clinical trials [3, 4]. While about 8% of patients receive anti-programmed death-1 (PD-1) monotherapy, up to 50% are treated with combination ICIs and chemotherapy as first-line therapy [5]. Most patients eventually experience tumor progression after first-line ICI-based treatment, necessitating subsequent therapeutic options [6].

Several ICIs have been investigated as second-line or subsequent treatments for patients with advanced ESCC who have not previously received immunotherapy [7, 8]. The ATTRACTION-

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03 trial found that the median OS for the nivolumab group was 10.9 months, 2.5 months longer than the chemotherapy group (8.4 months), with a 23% reduced risk of death [9, 10]. The KEYNOTE-181 study showed that pembrolizumab significantly prolonged OS in patients with a PD-L1 combined positive score (CPS) ≥10 compared to chemotherapy [11]. However, these trials excluded patients who had previously received ICIs, limiting their applicability in the current clinical scenario, where first-line ICI-based therapy is now standard.

In real-world clinical practice, patients who progress after first-line ICI therapy have limited treatment options [12]. Systemic treatments such as tyrosine kinase inhibitors (TKIs) and conventional chemotherapy are often empirically used, but their efficacy is restricted [13]. Given the dynamic nature of immune responses and the potential for renewed immune activation, ICI rechallenge - defined as the readministration of ICIs to patients who have progressed after ICI-based regimens - appears to be a promising therapeutic approach [14-16]. This strategy may involve either retreatment with the same ICI or crossline treatment with a different ICI agent.

The biological rationale for ICI rechallenges is supported by several mechanisms. First, the tumor immune microenvironment may evolve during treatment-free intervals, potentially restoring immune responsiveness [17]. Second, acquired resistance to initial ICI therapy could be overcome through different immune pathways or combination strategies [18-20]. Third, patients who initially benefited from ICI therapy may retain immune memory that can be reactivated with rechallenge [20].

We define first-line therapy as the initial systemic treatment for advanced or metastatic ESCC, specifically consisting of ICI (anti-PD-1/PD-L1 antibodies) combined with chemotherapy or ICI monotherapy. ICI rechallenge refers to the administration of any ICI agent as second-line therapy to patients who have previously received and progressed on first-line ICI-based regimens [20]. This rechallenge strategy can be further categorized into retreatment, where the same ICI agent used in first-line therapy is readministered, or crossline treatment, where a different ICI agent is employed. Patients were stratified based on first-line treatment duration, with those receiving ICI-based therapy for at least 180 days before disease progression considered to have demonstrated initial ICI sensitivity.

While clinical evidence has supported the feasibility of ICI rechallenge in ESCC, comprehensive real-world data on rechallenge strategies, optimal patient selection, and predictive biomarkers are still lacking. Several critical questions regarding ICI rechallenge remain unanswered: Which patients are most likely to benefit from rechallenge therapy? Should rechallenge involve the same ICI or a different agent? What is the optimal combination strategy for rechallenge (monotherapy vs. combination with chemotherapy or antiangiogenic agents)? How does the duration of initial ICI benefit influence rechallenge outcomes? What is the safety profile of ICI rechallenge, particularly regarding immune-related adverse events (irAEs)?

Therefore, we conducted this retrospective multicenter study to address these critical questions by analyzing real-world data from patients with ESCC who received ICI rechallenge therapy after progression on first-line ICI-based treatment. We specifically evaluated the efficacy and safety of second-line ICI rechallenge, identified predictive factors for treatment response, and compared different rechallenge strategies to provide evidence-based guidance for clinical practice.

Materials and methods

Study design and ethics

This retrospective study was approved by the Research Ethics Committee of the First People's Hospital of Jingdezhen (ID number: jdzyy202505). The study adhered to the Declaration of Helsinki (revised in 2013). As an observational retrospective study conducted within a routine medical setting, it did not involve additional interventions or risks to patients. Therefore, informed consent was waived by the ethics committee.

Patient selection and data collection

We conducted a retrospective analysis of patients with histologically confirmed ESCC treated from December 2021 to November 2024 across multiple centers. The detailed patient screening and selection flowchart is presented in Figure S1. Patients were eligible

for inclusion if they had histologically confirmed esophageal squamous cell carcinoma, were aged 18 years or older, had unresectable advanced, metastatic, or recurrent disease, and had previously received first-line ICI-based therapy (anti-PD-1/PD-L1 antibodies combined with chemotherapy or ICI monotherapy) for advanced or metastatic disease. Additional inclusion criteria required radiologically confirmed disease progression (via computed tomography or magnetic resonance imaging) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 before initiating second-line ICI treatment. Patients must have received at least 4 cycles of both first-line and second-line ICI-based therapy, unless interrupted by disease progression, unacceptable toxicity, or patient or physician decision. Eligible patients had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 to 2, expected survival of more than 3 months at second-line treatment initiation, and adequate organ function (hepatic, renal, and hematologic parameters within acceptable ranges for ICI treatment).

Patients were excluded if they had unclear or unconfirmed pathological diagnoses, lacked documented first-line or second-line ICI-based therapy, were enrolled in interventional clinical trials, had active autoimmune diseases requiring systemic immunosuppressive therapy, had a history of organ transplantation, had concurrent active malignancies other than ESCC, or had insufficient follow-up data (less than 3 months), unless death occurred.

Treatment protocols and drug specifications

First-line therapy was defined as the initial systemic treatment for advanced or metastatic ESCC. ICI monotherapy regimens included pembrolizumab 200 mg every 3 weeks intravenously, camrelizumab 200 mg every 3 weeks intravenously, sintilimab 200 mg every 3 weeks intravenously, or toripalimab 240 mg every 3 weeks intravenously. ICI combined with chemotherapy regimens consisted of any of the above ICI agents plus paclitaxel 175 mg/m^2 every 3 weeks and cisplatin 75 mg/m^2 every 3 weeks, or ICI plus paclitaxel 175 mg/m^2 every 3 weeks and carboplatin AUC 5 to 6 every 3 weeks, or ICI plus docetaxel 75 mg/m^2 every 3 weeks and cisplatin 75 mg/m^2 every 3 weeks.

Second-line ICI rechallenge therapy was categorized into four distinct strategies: (1) Immunotherapy alone: This included continuing the same ICI agent used in first-line treatment at the same dose and schedule, or switching to an alternative anti-PD-1 or PD-L1 antibody at standard dosing. (2) Immunotherapy plus chemotherapy: This included ICI combined with single-agent chemotherapy such as paclitaxel 80 to 100 mg/m^2 weekly, docetaxel 75 mg/m^2 every 3 weeks, or irinotecan 125 to 180 mg/m^2 every 2 weeks, or ICI combined with combination chemotherapy regimens such as FOLFIRI (leucovorin 400 mg/m^2, fluorouracil 400 mg/m^2 bolus, and 2,400 mg/m^2 continuous infusion over 46 hours, plus irinotecan 180 mg/m^2 every 2 weeks) or other physician-selected regimens. (3) Immunotherapy plus antiangiogenic therapy: This involved ICI combined with apatinib 250 to 425 mg daily orally, bevacizumab 7.5 mg/kg every 3 weeks intravenously, or anlotinib 10 to 12 mg daily orally (14 days on, 7 days off). (4) Immunotherapy plus chemotherapy plus antiangiogenic therapy: This represented combinations of the above strategies based on physician discretion and patient tolerance.

The same immune drug group comprised patients who continued with the same anti-PD-1 or PD-L1 antibody used in first-line therapy for second-line treatment, regardless of combination partners. The different immune drug group included patients who switched to a different anti-PD-1 or PD-L1 antibody for second-line treatment compared to first-line therapy.

Data collection

The data in this paper are sourced from Jingdezhen First People's Hospital, Jiangxi Provincial People's Hospital (the First Affiliated Hospital of Nanchang Medical College), and the Cangshan Campus of the 900th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army. Clinical characteristics collected included demographics such as age, sex, and smoking history (classified as never, former, or current smokers). Disease characteristics encompassed primary tumor location, histological grade, and the number and sites of metastatic lesions. Performance status was assessed using the ECOG PS scale at baseline and before second-line treatment. Biomarker status included PD-L1 expression determined by immunohistochemistry using the 22C3 pharmDx assay from Dako/Agilent, with combined positive score reported as <1% or ≥1%. Treatment details captured specific ICI

agents, dosing regimens, cycles administered, combination partners, dose modifications, and reasons for treatment discontinuation. Response assessments documented the best overall response for both first-line and second-line treatments according to RECIST version 1.1. Survival data included progression-free survival during first-line treatment, progression-free survival during second-line treatment, and overall survival. Safety data comprised irAEs graded according to Common Terminology Criteria for Adverse Events version 5.0.

Outcome measures

The primary outcomes were progression-free survival (PFS) during second-line treatment, defined as the time from initiation of second-line ICI therapy to the first documented disease progression according to RECIST version 1.1 or death from any cause, whichever occurred first, and overall survival (OS), measured as the time from the date of initial diagnosis of advanced or metastatic ESCC to death from any cause. Secondary outcomes included progression-free survival during first-line treatment, defined as the time from initiation of first-line ICI therapy to the first documented disease progression according to RECIST version 1.1 or death from any cause, whichever occurred first. Additional secondary endpoints encompassed objective response rate (ORR), calculated as the proportion of patients achieving complete response (CR) or partial response (PR) according to RECIST version 1.1, disease control rate (DCR), representing the proportion of patients achieving CR, PR, or stable disease (SD) lasting 8 weeks or longer, and durable clinical benefit (DCB), defined as the proportion of patients achieving CR, PR, or SD lasting 6 months or longer. Safety endpoints included the incidence of all-grade and high-grade irAEs, with high-grade defined as grade 3 or higher according to Common Terminology Criteria for Adverse Events version 5.0, during both first-line and second-line ICI treatments.

Response assessment and follow-up

Tumor response was assessed by imaging techniques including computed tomography, magnetic resonance imaging, bone scintigraphy, or ultrasound, as clinically indicated, according to RECIST version 1.1. Imaging was performed every 6 to 9 weeks, corresponding to every 2 to 3 treatment cycles during active treatment, and every 3 months during follow-up until disease progression or death. Response evaluations were conducted by treating physicians and confirmed by institutional radiologists when available. Patients were followed until death, loss to follow-up, or data cutoff on January 31, 2025. Patients alive without progression at the last follow-up were censored for survival analyses.

Statistical analysis

All statistical analyses were performed using IBM SPSS version 26.0. Descriptive statistics were reported as medians with interquartile ranges for continuous variables and frequencies with percentages for categorical variables. Continuous variables were compared using the two-sided Mann-Whitney U test, while categorical variables were analyzed using the chi-squared test. The Kaplan-Meier method was used to estimate survival curves for overall survival and progression-free survival during second-line treatment, with differences between groups assessed using the log-rank test. Hazard ratios with 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression.

For pairwise comparisons among the four second-line treatment regimens, Bonferroni correction was applied to control for multiple testing, with the adjusted significance level set at $P<0.0083$ (calculated as 0.05 divided by 6 comparisons). Cox proportional hazards regression models were used to identify independent predictors of progression-free survival during second-line treatment and overall survival. Variables with $P<0.10$ in univariate analysis or those considered clinically relevant were included in the multivariable models. These variables included age (analyzed both continuously and categorized as <65 vs. ≥65 years), sex, ECOG PS (categorized as 0 to 1 vs. 2), smoking history (classified as never vs. ever), number of metastatic sites (grouped as 0 to 2 vs. ≥3), PD-L1 expression status (combined positive score <1% vs. ≥1%), first-line treatment duration (PFS <180 vs. ≥180 days), best response to first-line treatment (grouped as CR, PR, or SD vs. progressive disease), second-line treatment regimen (compared across immunotherapy alone, immunotherapy plus chemotherapy, immunotherapy plus antiangiogenic therapy, and immunotherapy plus chemotherapy plus antiangiogenic therapy), and ICI drug consistency (comparing same vs. different agents).

Table 1. Patient characteristics

Variables (n = 171)	PFS1 180 days (1st line)			Immune drugs (2nd line)			Best response (2nd line ICI)		
	Less n = 84 (%)	More n = 87 (%)	p	Same n = 82 (%)	Different n = 89 (%)	p	PR/SD n = 133 (%)	PD n = 38 (%)	p
Age (median IQR), y*	65 (61-70)	66 (62-71)	0.876	62 (60-70)	66 (62-70)	0.587	66 (62-70)	65 (60-70)	0.681
Sex			0.146			0.087			0.434
Male (n = 86)	47 (76.2)	39 (67.8)		40 (62.2)	56 (69.7)		77 (74.4)	19 (63.2)	
Female (n = 85)	37 (23.8)	48 (32.2)		42 (37.8)	33 (30.3)		56 (25.6)	19 (36.8)	
ECOG			0.093			0.795			0.582
0-1 (n = 149)	73 (86.9)	76 (87.4)		69 (84.1)	80 (89.9)		114 (85.7)	35 (92.1)	
2 (n = 22)	11 (13.1)	11 (12.6)		13 (15.9)	9 (10.1)		19 (14.3)	3 (7.9)	
Metastatic number†			0.417			0.845			0.263
2 (n = 112)	55 (65.5)	57 (65.5)		56 (68.3)	56 (62.9)		83 (62.4)	29 (76.3)	
3 (n = 34)	14 (16.7)	20 (23.0)		17 (20.7)	17 (19.1)		29 (21.8)	5 (13.2)	
PD-L1 expression†			0.172			0.496			0.437
CPS <1% (n = 22)	22 (26.2)	0 (0)		9 (11.0)	13 (14.6)		0 (0)	22 (57.9)	
CPS ≥1% (n = 61)	53 (63.1)	8 (9.2)		32 (39.0)	29 (32.6)		45 (33.8)	16 (42.1)	
Regimens in 2nd line therapy			<0.001			0.668			0.405
I (n = 60)	17 (20.2)	4 (49.4)		28 (34.1)	32 (36.0)		49 (18.9)	11 (28.9)	
I+C (n = 57)	40 (47.6)	17 (19.5)		27 (32.9)	30 (33.7)		40 (36.8)	17 (44.7)	
I+A (n = 36)	14 (16.7)	22 (25.4)		20 (24.4)	16 (18.0)		29 (30.1)	7 (18.5)	
I+A+C (n = 18)	13 (15.5)	5 (5.7)		7 (8.6)	11 (12.3)		15 (14.2)	3 (7.9)	
Best response to 1st-line ICI			0.947			0.191			0.004
CR/PR/SD (n = 158)	78 (92.9)	80 (92.0)		73 (89.1)	85 (95.5)		127 (95.5)	31 (81.6)	
PD (n = 13)	6 (7.1)	7 (8.0)		9 (10.9)	4 (4.5)		6 (4.5)	7 (18.4)	
Best response to 2nd-line ICI			<0.001			0.934			<0.001
PR/SD (n = 133)	48 (57.1)	85 (97.7)		64 (78.0)	69 (77.5)		133 (100)	0 (0)	
PD (n = 38)	36 (42.9)	2 (2.3)		18 (22.0)	20 (22.5)		0 (0)	38 (100)	
PFS1			<0.001			0.315			<0.001
<180 days (n = 84)	84 (100)	0 (0)		37 (45.1)	47 (52.8)		48 (36.1)	36 (94.7)	
≥180 days (n = 87)	0 (0)	87 (100)		45 (54.9)	42 (47.2)		85 (63.9)	2 (5.3)	
2nd line immune drugs			0.315			<0.001			0.935
Same (n = 82)	37 (44.0)	45 (51.7)		82 (100)	0 (0)		64 (48.1)	18 (47.4)	
Different (n = 89)	47 (56.0)	42 (48.3)		0 (0)	89 (100)		69 (51.9)	20 (52.6)	

Note: *Comparison of data using the Mann-Whitney U-test; Other data comparison using the two-sided Chi-square or Fisher's exact test. †Number of cases available. Abbreviations: PFS, progress-free survival; IQR, interquartile range; ICI, immune checkpoint inhibitor; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death-ligand 1; CPS, combined positive score; SD, stable disease; PR, partial response; PD, progressive disease; CR, complete response.

The proportional hazards assumption was tested using Schoenfeld residuals, and model selection was performed using backward elimination with a significance level of P<0.05 for retention in the final model. All Kaplan-Meier survival curves included 95% CIs, and effect sizes for survival outcomes are reported as hazard ratios with 95% CIs. For categorical outcomes, odds ratios with 95% CIs were calculated where appropriate. Missing data patterns were assessed and reported, with patients having missing outcome data censored at the last known follow-up date for survival analyses. Sensitivity analyses were performed to assess the impact of missing data on primary conclusions. Statistical significance was defined as two-sided P<0.05 for primary analyses and P<0.0083 for multiple comparisons after Bonferroni correction. All analyses were exploratory, and no formal sample size calculation was performed for this retrospective study.

Results

Patient pathologic characteristics

Table 1 presents the patient demographics, with a male-to-female ratio of 1:1.25 (95 males, 76 females) and a median age of 66 years (interquartile range: 61-70). Patients were stratified into two groups based on PFS1 duration: ≥180 days (n = 87, 50.9%) and <180 days (n = 84, 49.1%). Additionally, patients were catego-

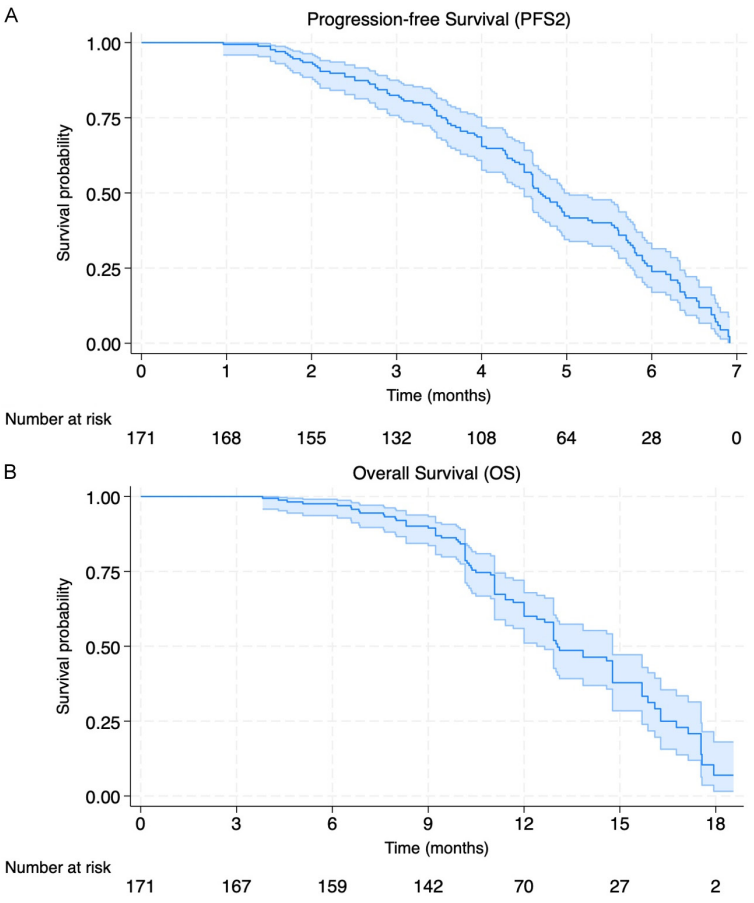

Figure 1. Survival Outcomes in the Overall Cohort. Kaplan-Meier survival curves for the entire study population (n = 171). A. Progression-free survival during second-line treatment (PFS2) showing a median PFS2 of 4.69 months (95% CI: 4.45-4.92). B. Overall survival (OS) demonstrating a median OS of 13.04 months (95% CI: 12.09-14.01) with a median follow-up of 14.68 months (95% CI: 13.46-15.89). Shaded areas represent 95% confidence intervals.

rized by second-line immune therapy use (same as first-line: n = 82, 47.9%; different: n = 89, 52.1%) and clinical efficacy of second-line treatment (PR/SD: n = 133, 77.8%; PD: n = 38, 22.2%).

Among patients with PFS1 ≥180 days, a higher proportion received immune monotherapy as second-line treatment (P<0.001) and achieved PR or SD (P<0.001). Patients with PR/SD to second-line treatment were more likely to have had PR/SD to first-line treatment (P<0.001). No significant differences were observed in second-line immune drug choice or across age, gender, ECOG PS, number of metastases, and PD-L1 expression levels (all P>0.05). Detailed patient characteristics are provided in **Table 1**.

PFS2 and OS based on PFS1 duration

The pooled cohort exhibited a median PFS2 of 4.69 months (95% CI: 4.45-4.92) (**Figure 1A**) and a median OS of 13.04 months (95% CI: 12.09-14.01) with a median follow-up of 14.68 months (95% CI: 13.46-15.89) (**Figure 1B**). Among the patients, 89 (52.05%) succumbed to the disease, and tumor progression was observed in 133 patients (77.78%).

For patients with a first-line treatment duration exceeding 180 days, the median PFS2 was 5.70 months (95% CI: 5.49-5.91), whereas for those with a shorter duration, it was 3.47 months (95% CI: 2.79-4.15; P<0.001) (**Figure 2A**). Specifically, in the <180-day group, the 3- and 6-month PFS2 rates were 53.57% and 7.14%, respectively. Conversely, in the ≥180-day group, these rates were 100% and 25.29%, respectively. Patients with PFS1 <180 days had a median OS of 12.92 months (95% CI: 11.75-14.10), compared to 14.77 months (95% CI: 12.94-16.60) for those with longer PFS1 (P = 0.008) (**Figure 2B**). The 6-, 12-, and 18-month OS rates were 86.90%, 35.71%, and 0%, respectively, in the <180-day group, and 98.85%, 45.98%, and 2.29%, respectively, in the ≥180-day group.

Stratified survival analysis controlling for confounding factors

Stratified log-rank tests were performed to account for potential confounding factors. The survival advantage for patients with PFS1 ≥180 days remained statistically significant after stratification by metastatic burden (P<0.001), ECOG PS (P<0.001), and age group (P<0.001). When stratified by multiple factors simultaneously (metastatic burden and ECOG PS), the difference remained highly significant (P<0.001), with a chi-square value of 30.05, indicating

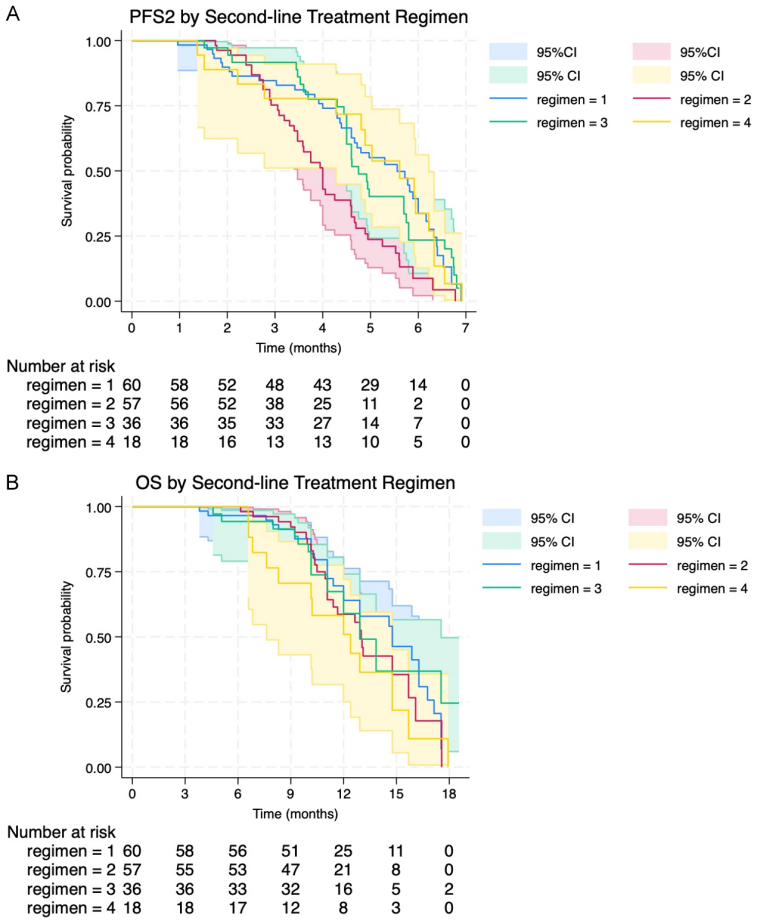

Figure 2. Impact of First-line Treatment Duration on Second-line Outcomes. Kaplan-Meier survival curves stratified by first-line progression-free survival (PFS1) duration. A. PFS2 comparison between patients with PFS1 ≥180 days (n = 87) versus <180 days (n = 84), showing median PFS2 of 5.70 months (95% CI: 5.49-5.91) versus 3.47 months (95% CI: 2.79-4.15), respectively (P<0.001). B. Overall survival comparison demonstrating median OS of 14.77 months (95% CI: 12.94-16.60) for PFS1 ≥180 days versus 12.92 months (95% CI: 11.75-14.10) for PFS1 <180 days (P = 0.008). The 180-day cutoff represents a critical predictor of rechallenge efficacy.

robust statistical significance independent of these confounding factors. For overall survival, stratified analyses also confirmed the prognostic value of PFS1 duration ≥180 days when controlling for metastatic burden (P = 0.007), ECOG PS (P = 0.005), and age group (P = 0.017), demonstrating that the survival benefit persists across different patient subgroups.

PFS2 and OS by second-line treatment regimens

The median PFS2 for patients receiving immunotherapy alone (I), immunotherapy plus chemotherapy (I+C), immunotherapy plus antiangiogenic drugs (I+A), and immunotherapy plus antiangiogenic drugs and chemotherapy (I+A+C) as second-line treatments were 5.56 months (95% CI: 4.50-6.23), 4.00 months (95% CI: 3.62-4.38), 4.75 months (95% CI: 4.33-5.17), and 5.61 months (95% CI: 4.34-6.89), respectively (P<0.001; **Figure 3A**).

After applying Bonferroni correction for multiple comparisons (adjusted significance level P<0.0083), the I+C regimen exhibited significantly shorter PFS2 compared to I alone (HR 2.137, 95% CI: 1.375-3.321, P = 0.001), I+A (HR 1.871, 95% CI: 1.162-3.011, P = 0.008), and I+A+C (HR 2.122, 95% CI: 1.142-3.942, P = 0.014). However, the PFS2 differences among I alone, I+A, and I+A+C regimens were not statistically significant after correction for multiple comparisons.

The median OS for the I, I+C, I+A, and I+A+C groups were 14.77 months (95% CI: 11.79-17.75), 13.00 months (95% CI: 12.42-13.58), 13.00 months (95% CI: 11.31-14.53), and 12.40 months (95% CI: 8.66-16.12), respectively (P = 0.325; **Figure 3B**). No statistically significant differences in OS were observed among the four treatment regimens, even after Bonferroni correction (P>0.05).

PFS2 and OS by second-line ICI drug consistency

For patients in the second-line therapy cohort, the median PFS2 was 5.25 months (95% CI: 4.51-5.99) among those who maintained the same immune medications and 4.60 months (95% CI: 4.36-4.85, P = 0.152) for those who changed their immune drugs. Patients in the same immune medication group had a median OS of 14.77 months (95% CI: 12.83-16.71), and those in the different immune medication

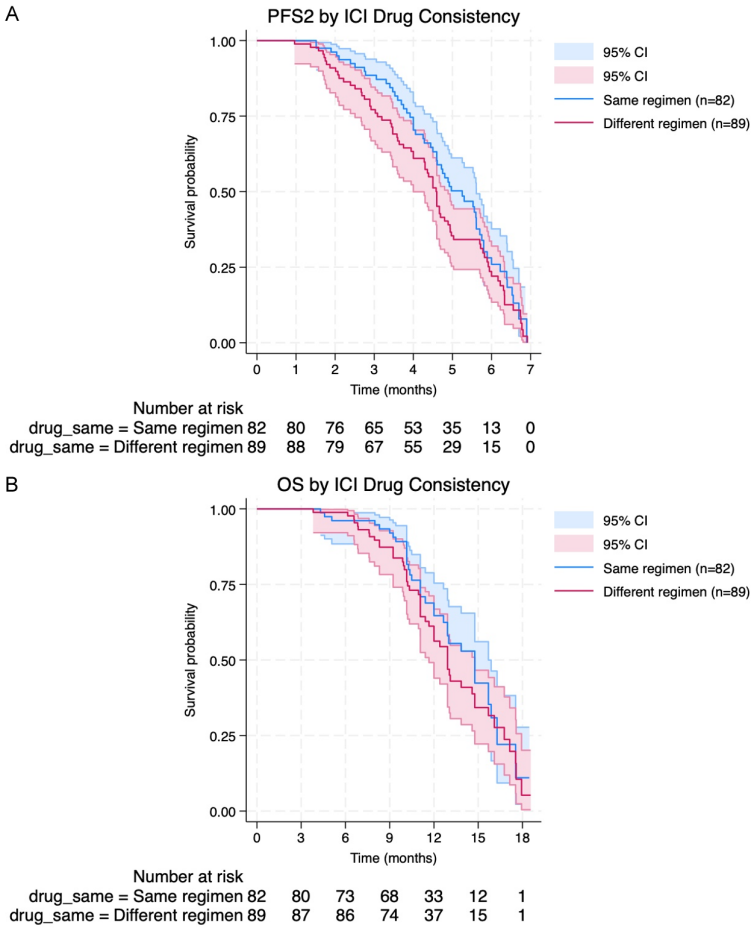

Figure 3. Efficacy of Different Second-line Treatment Regimens. Kaplan-Meier survival curves comparing four second-line treatment strategies. A. PFS2 analysis showing median values of 5.56 months for immunotherapy alone (I), 4.00 months for immunotherapy plus chemotherapy (I+C), 4.75 months for immunotherapy plus antiangiogenic therapy (I+A), and 5.61 months for triple combination (I+A+C) (P<0.001). After Bonferroni correction, I+C showed significantly inferior PFS2 compared to other regimens. B. Overall survival comparison revealing no statistically significant differences among treatment regimens (P = 0.325), with median OS ranging from 12.40 to 14.77 months across groups.

group had a median OS of 12.92 months (95% CI: 11.79-14.05, P = 0.346). No statistically significant differences were observed in either PFS2 or OS between patients who continued the same ICI versus those who switched to different agents (both P>0.05).

PD-L1 expression and survival correlation

Among the 83 patients with known PD-L1 status (excluding 88 patients with unknown status), 22 (26.5%) had CPS <1% and 61 (73.5%) had CPS ≥1%. PD-L1 expression demonstrated a significant association with PFS2 outcomes. Patients with CPS <1% had markedly superior PFS2 compared to those with CPS ≥1% (P<0.001), with the PD-L1 negative group showing prolonged disease control. However, no significant difference in overall survival was observed between PD-L1 expression groups (P = 0.495).

In Cox regression analysis, PD-L1 CPS ≥1% was associated with significantly increased risk of disease progression during second-line treatment. However, PD-L1 expression was not an independent predictor of overall survival (HR 0.805, 95% CI: 0.424-1.529, P = 0.507).

irAEs and clinical response correlation

The pooled incidence of all-grade and high-grade irAEs during second-line ICI treatment reached 32.2% and 14.0%, respectively (**Table 2**). Rechallenge did not significantly alter the incidence of all-grade or high-grade irAEs compared to initial ICI treatment (P = 0.435). Hypothyroidism (22 cases, 12.9%) and dermatitis (15 cases, 8.8%) were the predominant irAEs during second-line treatment (**Table 2**).

Importantly, the clinical benefit response associated with irAEs was significantly reduced during rechallenge compared to first-line treatment (P<0.001). Among patients who experienced irAEs during second-line treatment, the objective response rate and disease control rate were not significantly different from those without irAEs, in contrast to the well-established positive correlation observed during first-line ICI therapy. This suggests a potential decoupling of irAE occurrence from therapeutic efficacy during ICI rechallenge.

Multivariable analysis for independent predictors

In comprehensive multivariable Cox regression analysis, three independent predictors of PFS2

Table 2. IrAE characteristics

Characteristics	Initial irAEs	Sequential irAEs	*P* value
IrAE events, n (%)	49 (28.7%)	55 (32.4%)	0.435
Type of irAE, n (%)			0.719
Myocarditis	2 (1.2%)	0 (0)	
Bullous pemphigoid	1 (0.6%)	0 (0)	
Colitis	4 (2.3%)	3 (1.8%)	
Conjunctivitis	3 (1.8%)	2 (1.2%)	
Dermatitis	12 (7.0%)	15 (8.8%)	
Hypothyroidism	18 (10.5%)	22 (12.9%)	
Pneumonitis	9 (5.3%)	13 (7.6%)	
Grade of irAE, n (%)			0.508
1	22 (12.9%)	20 (11.7%)	
2	11 (6.4%)	11 (6.4%)	
3	16 (9.4%)	24 (14%)	
Clinical response to ICI, n (%)			<0.001
PD	1 (0.6%)*	11 (6.4%)◇	
SD	31 (18.1%)*	39 (22.8%)◇	
PR/ORR	17 (9.9%)*	5 (2.9%)◇	

Note: *The response in first-line ICI treatment; ◇The response in second-line ICI retreatment; Abbreviations: irAE, immune-related adverse events; PD, progressive disease; SD, stable disease; PR, partial response; ORR, objective response rate.

were identified among the 83 patients with known PD-L1 status (**Figure 4**). First-line treatment duration ≥180 days remained a strong independent predictor of improved PFS2 (HR 1.689, 95% CI: 0.668-4.273, P = 0.268, though not statistically significant in this subset). The use of different ICI drugs in second-line treatment was associated with improved PFS2 (HR 2.290, 95% CI: 1.325-3.955, P = 0.003). Additionally, ECOG PS 3 showed a trend toward worse prognosis (HR 7.524, 95% CI: 0.859-65.911, P = 0.068). For overall survival, no independent predictors reached statistical significance in the multivariable model, likely due to the reduced sample size when excluding patients with unknown PD-L1 status and the relatively immature survival data.

Clinical response evaluation

Table 3 presents the patient counts and variations in optimal clinical responses among groups. Patients with PFS1 ≥180 days exhibited significantly higher proportions in PR (n = 13 vs. 0, P<0.001), stable disease (n = 72 vs. 48, P = 0.004), disease control rate (n = 85 vs. 48, P = 0.01), objective response rate (n = 13 vs. 0, P<0.001), and durable clinical benefit (n = 22 vs. 6, P<0.001) compared to those with PFS1 <180 days.

Regarding second-line regimens, durable clinical benefit counts varied significantly across I, I+C, I+A, and I+A+C groups (14, 7, 2, and 5, respectively; P = 0.012), whereas no statistically significant differences were observed in PR, SD, DCR, or ORR proportions. Furthermore, clinical responses were unrelated to whether second-line immune therapies matched first-line treatments, confirming that drug consistency does not significantly impact treatment efficacy in the rechallenge setting.

Discussion

This multicenter retrospective study provides comprehensive real-world evidence on the efficacy and safety of ICI rechallenge in patients with advanced ESCC who progressed after first-line ICI-based therapy. Our findings show that ICI rechallenge is a viable therapeutic strategy with promising clinical outcomes, particularly for patients who initially demonstrated prolonged benefit from first-line immunotherapy.

The median PFS2 of 4.69 months and median OS of 13.04 months observed in our cohort compare favorably with historical controls and recent clinical studies. Our study supports these findings while providing additional insights into the biological and clinical factors influencing rechallenge efficacy.

A key finding of our analysis is the robust predictive value of first-line treatment duration ≥180 days for rechallenge outcomes. Patients who received first-line ICI-based therapy for at least 6 months demonstrated significantly superior PFS2 and OS. Importantly, this survival advantage remained statistically significant even after controlling for potential confounding factors, including metastatic burden, ECOG PS, and age, through stratified log-rank analyses. This suggests that prolonged initial ICI benefit reflects intrinsic tumor biology and immune microenvironment characteristics that favor sustained immune activation upon rechallenge.

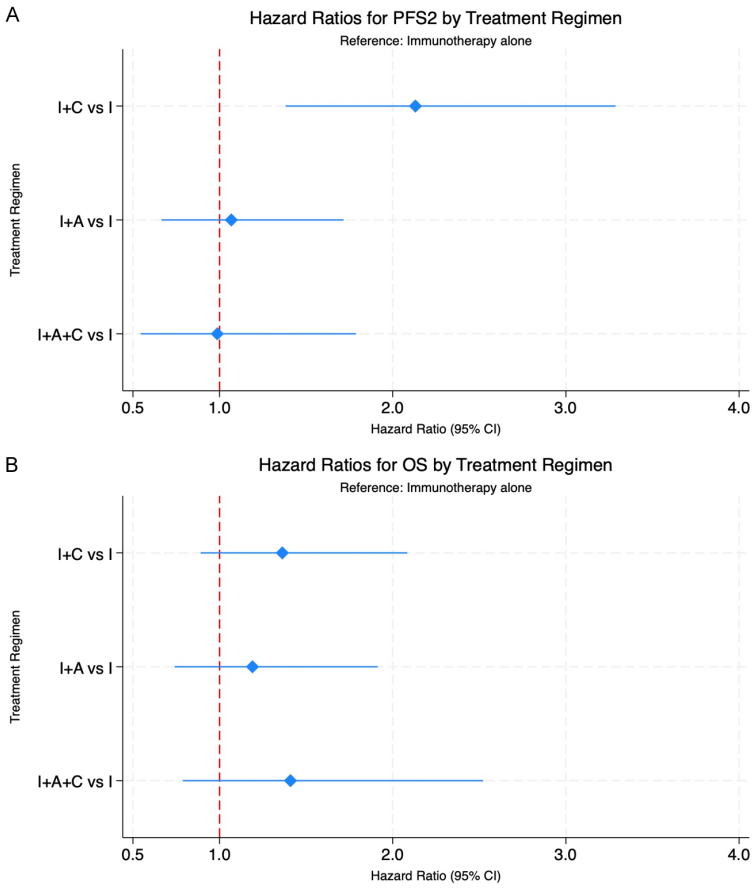

Figure 4. Multivariable Cox Regression Analysis of Factors Associated with Second-line Treatment Outcomes. Forest plots showing hazard ratios (HR) and 95% confidence intervals from multivariable Cox regression analysis. A. Factors associated with progression-free survival during second-line treatment (PFS2), demonstrating that different ICI drugs (HR 2.290, 95% CI: 1.325-3.955, P = 0.003) and first-line treatment duration ≥180 days (HR 1.689, 95% CI: 0.668-4.273, P = 0.268) were key predictive factors among patients with known PD-L1 status (n = 83). B. Factors associated with overall survival, showing that no independent predictors reached statistical significance in the multivariable model, likely due to reduced sample size and relatively immature survival data. The vertical dashed line represents HR = 1.0 (no effect), with points to the left favoring better outcomes and points to the right indicating worse prognosis.

The biological rationale for this observation likely relates to immune memory and the heterogeneous nature of acquired resistance to ICIs. Patients who initially benefit from prolonged ICI therapy may harbor tumors with preserved immune infiltration, lower mutational burden heterogeneity, or less aggressive resistance mechanisms compared to those who rapidly progress. Furthermore, the treatment-free interval between first-line progression and second-line initiation may allow for immune system recovery and restoration of anti-tumor immune responses, particularly in patients who initially demonstrated immune sensitivity.

When compared to the landmark ATTRACTION-3 trial, which established nivolumab as standard second-line therapy for ICI-naive ESCC patients, our rechallenge cohort demonstrated comparable survival outcomes despite the inherently more challenging patient population [10]. The ATTRACTION-3 study reported a median OS of 10.9 months with nivolumab versus 8.4 months with chemotherapy in patients who had not previously received ICIs [10]. Our median OS of 13.04 months in the rechallenge setting suggests that selected patients may derive substantial benefit from continued immunotherapy rather than switching to conventional chemotherapy or targeted agents.

This comparison highlights an important clinical consideration: while ATTRACTION-3 and similar pivotal trials specifically excluded patients with prior ICI exposure, the current treatment landscape requires evidence-based strategies for the growing population of patients who progress after first-line immunotherapy. Our study fills this critical knowledge gap by demonstrating that ICI rechallenge can achieve clinically meaningful outcomes in appropriately selected patients.

Our analysis revealed important differences among second-line treatment regimens, with the immunotherapy plus chemotherapy (I+C) combination showing inferior PFS2 compared to other approaches. After applying Bonferroni correction for multiple comparisons, I+C demonstrated significantly shorter median PFS2 compared to immunotherapy alone, immunotherapy plus antiangiogenic therapy, and triple combination therapy.

Table 3. Response to ICIs in 2nd-line immunotherapy

Response (n)	Immune drugs			Regimens					PFS1 (180 days)		
	Same	Different	p	I	I+C	I+A	I+A+C	p	Less	More	p
PD (n = 38)	17	19	0.977 (PD vs. SD)	11	17	7	3	0.457 (PD vs. SD)	36	2	<0.001 (PD vs. SD)
SD (n = 120)	57	63	0.644 (SD vs. PR)	41	37	27	15	0.211 (SD vs. PR)	48	72	0.004 (SD vs. PR)
PR (n = 13)	7	6	0.682 (PR vs. PD)	8	3	2	0	0.172 (PR vs. PD)	0	13	<0.001 (PR vs. PD)
ORR (n = 13)	7 (9%)	6 (7%)	0.712 (ORR vs. DCR)	8 (13%)	3 (5%)	2 (6%)	0	0.297 (ORR vs. DCR)	0	13(15%)	0.01 (ORR vs. DCR)
			0.657 (ORR vs. non-ORR)					0.222 (ORR vs. non-ORR)			<0.001 (ORR vs. non-ORR)
DCR (n = 133)	64 (79%)	68 (77%)	0.843 (DCR vs. DCB)	49 (82%)	40 (70%)	29 (76%)	15 (83%)	0.086 (DCR vs. DCB)	48 (57%)	85 (97%)	0.186 (DCR vs. DCB)
			0.893 (DCR vs. non-DCR)					0.405 (DCR vs. non-DCR)			<0.001 (DCR vs. non-DCR)
DCB (n = 28)	13	15	0.658 (DCB vs. ORR)	14	2	7	5	0.481 (DCB vs. ORR)	6	22	0.152 (DCB vs. ORR)
			0.836 (DCB vs. non-DCB)					0.012 (DCB vs. non-DCB)			0.001 (DCB vs. non-DCB)

Note: PFS, progress-free survival; PD-1, programmed death 1; SD, stable disease; PR, partial response; PD, progressive disease; CR, complete response; ORR, objective response rate; DCR, disease control rate; DCB, durable clinical benefit.

The inferior performance of I+C regimens in the rechallenge setting contrasts with the established benefit of immunochemotherapy combinations in treatment-naive patients. Several mechanisms may explain this paradox. First, chemotherapy-induced lymphodepletion and immune suppression may be more pronounced in patients with prior ICI exposure, potentially counteracting the immune-stimulating effects of checkpoint inhibition. Second, patients who progress after first-line immunochemotherapy may have developed cross-resistance mechanisms that affect both immune and cytotoxic pathways simultaneously. Third, the selection of chemotherapy agents in the rechallenge setting often involves drugs with different mechanisms of action compared to first-line therapy, which may not synergize optimally with ICIs in the context of acquired resistance.

Conversely, the addition of antiangiogenic agents to immunotherapy appeared to maintain efficacy, with I+A and I+A+C regimens showing comparable PFS2 to immunotherapy alone. This observation aligns with emerging evidence suggesting that VEGF pathway inhibition can enhance immune infiltration and overcome certain resistance mechanisms by normalizing tumor vasculature and reducing immunosuppressive factors within the tumor microenvironment.

One of the most intriguing findings of our study was the paradoxical relationship between PD-L1 expression and rechallenge efficacy. Contrary to expectations based on first-line ICI therapy data, patients with PD-L1 CPS <1% demonstrated significantly superior PFS2 compared to those with CPS ≥1%. However, this difference did not translate into an overall survival benefit, suggesting complex dynamics in the rechallenge setting.

Several hypotheses may explain this counterintuitive finding. First, PD-L1 expression patterns may evolve during first-line therapy and the treatment-free interval, with adaptive upregulation of PD-L1 potentially indicating more aggressive tumor biology or alternative resistance pathways rather than retained immune sensitivity. Second, patients with initially low PD-L1 expression who achieved prolonged disease control with first-line therapy may represent a biologically distinct subset with preserved immune surveillance mechanisms that remain responsive to rechallenge. Third, the predictive value of PD-L1 expression may differ fundamentally in the rechallenge setting compared to treatment-naive patients, requiring alternative biomarker strategies for patient selection.

This finding has important clinical implications and challenges the routine use of PD-L1 expression as a biomarker for ICI rechallenge decisions. Future studies should investigate dynamic changes in PD-L1 expression and explore alternative biomarkers, such as tumor mutational burden, immune infiltration patterns, and resistance pathway activation in the rechallenge setting.

Our analysis revealed a significant dissociation between irAEs and treatment efficacy during ICI rechallenge, representing a departure from established patterns observed in first-line therapy. While the overall incidence of irAEs remained comparable between first-line and rechallenge treatments, the positive correlation between irAE occurrence and clinical benefit was significantly attenuated during rechallenge.

This irAE-efficacy decoupling phenomenon may reflect several underlying mechanisms. First, prior ICI exposure may lead to immune system exhaustion or tolerance, reducing overall immune reactivity, which in turn affects both antitumor responses and autoimmune manifestations. Second, patients who tolerated first-line ICIs without significant irAEs may have intrinsic factors that limit both beneficial and detrimental immune activation upon rechallenge. Third, the use of immunosuppressive medications for first-line irAE management may have long-lasting effects on immune system function, which persist into the rechallenge period.

From a clinical perspective, this finding suggests that the absence of irAEs during rechallenge therapy should not be interpreted as a lack of treatment efficacy, contrary to conventional wisdom derived from first-line ICI studies. Healthcare providers should avoid premature treatment discontinuation based solely on the absence of immune-related toxicity during rechallenge therapy.

Contrary to some previous reports in other tumor types, our study found no significant difference in efficacy between patients who con-

tinued the same ICI agent and those who switched to different anti-PD-1/PD-L1 antibodies for rechallenge therapy. This suggests that the class effect of PD-1/PD-L1 inhibition may be more important than agent-specific characteristics in the rechallenge setting.

However, in multivariable analysis limited to patients with known PD-L1 status, the use of different ICI agents was associated with improved PFS2. This apparent contradiction may be explained by patient selection bias, differences in combination partners, or unmeasured confounding factors. The clinical significance of this finding requires validation in prospective studies with standardized treatment protocols.

Several limitations must be acknowledged in interpreting our results. First, the retrospective design introduces potential selection and information bias, though the multicenter approach and comprehensive data collection help mitigate these concerns. Second, the heterogeneity in first-line treatment regimens, rechallenge timing, and combination strategies reflects real-world clinical practice but may confound treatment comparisons. Additionally, the relatively short follow-up and immature survival data limit conclusions about long-term outcomes.

The high proportion of patients with unknown PD-L1 status (51.5%) represents a significant limitation that may affect the generalizability of our biomarker findings. This reflects the challenges of obtaining adequate tissue for biomarker testing in the advanced disease setting and highlights the need for liquid biopsy approaches or alternative tissue sampling strategies.

Our findings establish several important research priorities for optimizing ICI rechallenge strategies. First, prospective validation of the 180-day first-line duration cutoff as a predictive biomarker is essential, potentially through biomarker-driven clinical trials. Second, investigating dynamic biomarker changes during the treatment-free interval may identify additional predictive factors beyond static tissue-based markers. Third, exploring novel combination strategies that address acquired resistance mechanisms while minimizing overlapping toxicities is a critical research direction.

The development of liquid biopsy approaches for monitoring immune system evolution and resistance pathway activation during ICI rechallenge offers particular promise for precision medicine applications. Additionally, investigating the optimal timing for rechallenge initiation, duration of treatment-free intervals, and sequencing with other therapeutic modalities requires systematic study.

Based on our findings, we propose a clinical algorithm for ICI rechallenge decision-making in advanced ESCC. Patients who derived prolonged benefit (≥180 days) from first-line ICI-based therapy represent optimal candidates for rechallenge, regardless of PD-L1 expression status. For these patients, immunotherapy alone or in combination with antiangiogenic agents appears preferable to immunochemotherapy combinations. The absence of irAEs during rechallenge should not prompt treatment discontinuation in patients with stable disease or clinical benefit.

For patients with shorter first-line benefit (<180 days), ICI rechallenge may still be considered, but alternative treatment strategies or clinical trial participation should be prioritized. The decision should incorporate performance status, disease burden, and patient preferences while acknowledging the more limited expected benefit.

In conclusion, this comprehensive real-world analysis demonstrates that ICI rechallenge represents a feasible and effective treatment strategy for selected patients with advanced ESCC who progress after first-line immunotherapy. The duration of first-line ICI benefit emerges as the most robust predictive factor for rechallenge efficacy, while traditional biomarkers such as PD-L1 expression show paradoxical associations that require further investigation. The decoupling of irAEs from treatment efficacy during rechallenge represents an important clinical observation that should inform treatment monitoring strategies.

Our findings support the integration of ICI rechallenge into standard treatment algorithms for advanced ESCC while highlighting the need for biomarker-driven patient selection and optimized combination strategies. Future prospective studies should focus on validating predictive biomarkers, exploring novel combination approaches, and defining optimal treatment

sequencing to maximize the therapeutic potential of immunotherapy in this challenging patient population.

Disclosure of conflict of interest

None.

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References

[1] Li H, Wu H, Cao M, Yu Y, Zhou J, Zhang S, Tong F, Gong J, Wang H, Yang F, He S, Yan X, Zhang S, Luo P, Ma H, Liang L, Xia C and Chen W. Long-term incidence rates of esophageal squamous cell carcinoma in chinese patients with low-grade intraepithelial neoplasia and association of surveillance endoscopy with incidence. JAMA Network Open 2022; 5: e2247415.

[2] Nagami Y, Ominami M, Shiba M, Minamino H, Fukunaga S, Kameda N, Sugimori S, Machida H, Tanigawa T, Yamagami H, Watanabe T, Tominaga K, Fujiwara Y and Arakawa T. The five-year survival rate after endoscopic submucosal dissection for superficial esophageal squamous cell neoplasia. Dig Liver Dis 2017; 49: 427-33.

[3] Jiang M, Hu Y, Lin G, Chen C and Li H. Radiotherapy combined with immune checkpoint inhibitors in locally advanced/metastatic esophageal squamous cell carcinoma: clinical trials, efficacy and future directions. Front Immunol 2023; 14: 1177085.

[4] Vafaei S, Zekiy AO, Khanamir RA, Zaman BA, Ghayourvahdat A, Azimizonuzi H and Zamani M. Combination therapy with immune checkpoint inhibitors (ICIs); a new frontier. Cancer Cell Int 2022; 22: 2.

[5] Sun D, Ma J, Wang J, Wang L, Zhang S, Chen G, Li X, Cui P, Zheng X and Hu Y. A real-world study of the efficacy and safety of anti-programmed cell death-1 therapy combined with chemotherapy or targeted therapy in patients with advanced biliary tract cancer. J Gastrointest Oncol 2020; 11: 1421-1430.

[6] Hayashi H and Nakagawa K. Combination therapy with PD-1 or PD-L1 inhibitors for cancer. Int J Clin Oncol 2020; 25: 818-30.

[7] Zhu K, Chen H, Xu C, Chen D, Jin Z, Ren S, Witharana P, Chen B and Shen J. Efficacy and safety of immune checkpoint inhibitors versus chemotherapy in the second-line treatment of advanced esophageal squamous cell carcinoma: a meta-analysis and systematic review. J Thorac Dis 2023; 15: 1186-1195.

[8] Gómez de Liaño Lista A, van Dijk N, de Velasco Oria de Rueda G, Necchi A, Lavaud P, Morales-Barrera R, Alonso Gordoa T, Maroto P, Ravaud A, Durán I, Szabados B, Castellano D, Giannatempo P, Loriot Y, Carles J, Anguera Palacios G, Lefort F, Raggi D, Gross Goupil M, Powles T and Van der Heijden MS. Clinical outcome after progressing to frontline and second-line Anti-PD-1/PD-L1 in advanced urothelial cancer. Eur Urol 2020; 77: 269-76.

[9] Thuss-Patience P and Stein A. Immunotherapy in squamous cell cancer of the esophagus. Curr Oncol 2022; 29: 2461-71.

[10] Kato K, Cho BC, Takahashi M, Okada M, Lin CY, Chin K, Kadowaki S, Ahn MJ, Hamamoto Y, Doki Y, Yen CC, Kubota Y, Kim SB, Hsu CH, Holtved E, Xynos I, Kodani M and Kitagawa Y. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2019; 20: 1506-17.

[11] Kojima T, Shah MA, Muro K, Francois E, Adenis A, Hsu CH, Doi T, Moriwaki T, Kim SB, Lee SH, Bennouna J, Kato K, Shen L, Enzinger P, Qin SK, Ferreira P, Chen J, Girotto G, de la Fouchardiere C, Senellart H, Al-Rajabi R, Lordick F, Wang R, Suryawanshi S, Bhagia P, Kang SP and Metges JP; KEYNOTE-181 Investigators. Randomized phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. J Clin Oncol 2020; 38: 4138-48.

[12] Samuelly A, Di Stefano RF, Turco F, Delcuratolo MD, Pisano C, Saporita I, Calabrese M, Carfì FM, Tucci M and Buttigliero C. Navigating the ICI combination treatment journey: patterns of response and progression to first-line ICI-based combination treatment in metastatic renal cell carcinoma. J Clin Med 2024; 13: 307.

[13] Arteaga CL. Inhibiting tyrosine kinases: successes and limitations. Cancer Biol Ther 2003; 2 Suppl 1: S79-83.

[14] Hu H, Wang K, Jia R, Zeng ZX, Zhu M, Deng YL, Xiong ZJ, Tang JN, Xie H, Wang Y, Zhang P and Zhou J. Current status in rechallenge of immunotherapy. Int J Biol Sci 2023; 19: 2428-2442.

[15] Sun L, Cohen RB, D'Avella CA, Singh AP, Schoenfeld JD and Hanna GJ. Overall survival, treatment duration, and rechallenge outcomes with ICI therapy for recurrent or metastatic HNSCC. JAMA Netw Open 2024; 7: e2428526.

[16] Dolladille C, Ederhy S, Sassier M, Cautela J, Thuny F, Cohen AA, Fedrizzi S, Chrétien B, Da-

Silva A, Plane AF, Legallois D, Milliez PU, Lelong-Boulouard V and Alexandre J. Immune checkpoint inhibitor rechallenge after immune-related adverse events in patients with cancer. JAMA Oncol 2020; 6: 865-71.

[17] Pennock GK and Chow LQ. The evolving role of immune checkpoint inhibitors in cancer treatment. Oncologist 2015; 20: 812-22.

[18] Schoenfeld AJ and Hellmann MD. Acquired resistance to immune checkpoint inhibitors. Cancer Cell 2020; 37: 443-55.

[19] Marei HE, Hasan A, Pozzoli G and Cenciarelli C. Cancer immunotherapy with immune checkpoint inhibitors (ICIs): potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. Cancer Cell Int 2023; 23: 64.

[20] Plazy C, Hannani D and Gobbini E. Immune checkpoint inhibitor rechallenge and resumption: a systematic review. Curr Oncol Rep 2022; 24: 1095-106.

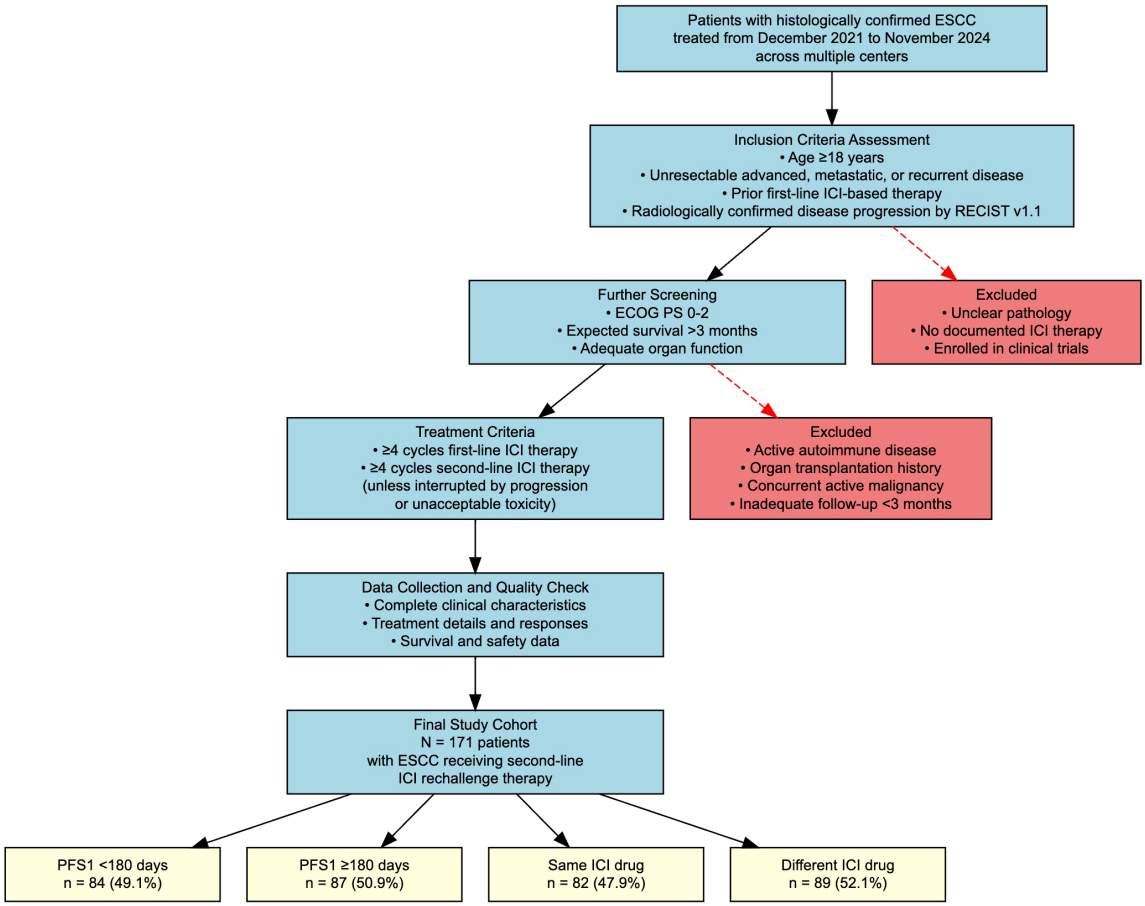

Figure S1. Patient selection flowchart for ESCC patients receiving second-line ICI rechallenge therapy. Flowchart showing the selection process for patients with histologically confirmed esophageal squamous cell carcinoma (ESCC) treated from December 2021 to November 2024 across multiple centers. Patients underwent screening based on inclusion criteria (age ≥18 years, unresectable advanced/metastatic/recurrent disease, prior first-line ICI therapy, radiologically confirmed disease progression by RECIST v1.1) and exclusion criteria. The final study cohort comprised 171 patients with ESCC receiving second-line ICI rechallenge therapy, stratified by progression-free survival from first-line therapy (PFS1 <180 days vs ≥180 days) and ICI drug selection (same vs different agent). ESCC, esophageal squamous cell carcinoma; ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group Performance Status; RECIST, Response Evaluation Criteria in Solid Tumors; PFS1, progression-free survival from first-line therapy.