Prognostic analysis and expression patterns of programmed cell death ligand 1 and BRAF V600E in esophageal neuroendocrine carcinoma

CHENGCHENG ZHANG[1], LI ZHENG[2], BINWEN XU[1], GUIDONG SHI[1] and MAOYONG FU[1]

[1]Department of Thoracic Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan 637000, P.R. China; [2]Department of Neurosurgery, The Third Hospital of Mianyang, Sichuan Mental Health Center, Mianyang, Sichuan 621000, P.R. China

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Abstract. Esophageal neuroendocrine carcinoma (ENEC) is a rare and highly aggressive malignancy. The present study investigated the expression of BRAF V600E and programmed death ligand 1 (PD-L1) and analyzed prognostic factors affecting patient outcomes. A total of 56 patients diagnosed with ENEC at the Affiliated Hospital of North Sichuan Medical College between January 2019, and September 2024 were retrospectively analyzed. Among them, 39 patients received surgery and were tested for BRAF V600E and PD-L1 expression using immunohistochemistry. Survival outcomes were estimated using the Kaplan-Meier method, and prognostic factors were assessed using Cox regression models. BRAF V600E mutations were identified in 12.8% (5/39) of cases, and PD-L1 expression was positive in 15.4% (6/39). In the mixed neuroendocrine carcinoma and squamous cell carcinoma (MiNEC-SCC), the positivity rates for both BRAF V600E and PD-L1 were 33.3% (3/9). Across the entire cohort, the 1-year overall survival (OS) and progression-free survival (PFS) rates were 79.7 and 43.1%, respectively, while the 3-year OS and PFS rates were 49.5 and 25.1%, respectively. Clinical lymph node stage and surgery were notably associated with both OS and PFS. Patients with tumor thickness >1 cm and length >3 cm had markedly worse PFS. Multivariate analysis identified surgery as an independent predictor of improved PFS (P=0.015). Among patients who underwent surgery, postoperative adjuvant therapy was independently associated with improved PFS (P=0.026). In conclusion, BRAF V600E and PD-L1 were more frequently expressed in the MiNEC-SCC compared with small cell neuroendocrine carcinoma. Surgery and postoperative adjuvant therapy were notably associated with improved PFS.

Correspondence to: Professor Maoyong Fu, Department of Thoracic Surgery, Affiliated Hospital of North Sichuan Medical College, 1 Maoyuan South Road, Nanchong, Sichuan 637000, P.R. China
E-mail: fumaoyongmd@163.com

Abbreviations: ENEC, esophageal neuroendocrine carcinoma; SCLC, small cell lung cancer; OS, overall survival; PFS, progression-free survival; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; SCNEC, small cell neuroendocrine carcinoma; MiNEC-SCC, mixed neuroendocrine carcinoma and squamous cell carcinoma; CI, confidence interval; GINEC, gastrointestinal neuroendocrine carcinoma

Key words: esophageal neuroendocrine carcinoma, esophageal small cell neuroendocrine carcinoma, programmed cell death ligand 1, BRAF V600E mutation, prognostic analysis

Introduction

Esophageal neuroendocrine carcinoma (ENEC) is a rare and highly aggressive extrapulmonary neuroendocrine malignancy, characterized by low incidence and notable challenges in clinical diagnosis and treatment. It is considered one of the most lethal forms of extrapulmonary neuroendocrine carcinoma, with globally reported median survival times ranging from 14 to 28 months (1-3). Even in patients diagnosed at a localized stage and treated with comprehensive multimodal therapy, the global 5-year overall survival (OS) rate remains as low as 8.2% (4). Owing to its rarity, large-scale, multicenter studies are lacking, and treatment strategies for ENEC remain controversial.

The RAS-RAF-MEK-ERK signaling cascade is one of the most critical oncogenic pathways involved in tumorigenesis (5). BRAF, as a key protein in this pathway, serves an essential role in the regulation of cellular proliferation, differentiation and apoptosis (6,7). Previous research has shown that dysregulation of this pathway is closely associated with the development and progression of neuroendocrine tumors, suggesting that BRAF inhibitors or RAF-1 activators may represent viable therapeutic targets (8). In colorectal neuroendocrine carcinomas, the prevalence of BRAF V600E mutations has been reported to range from 28 to 46.7% (9-11). However, the expression status and clinical implications of BRAF V600E mutations in ENEC remain unclear due to limited available data.

Programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) are critical immune checkpoints that regulate antitumor immunity and immune tolerance (12). In previous years, PD-1/PD-L1 inhibitors have demonstrated

notable therapeutic efficacy across various malignancies, including esophageal and lung cancers (13-16). Several clinical trials have demonstrated that the combination of chemotherapy with PD-1/PD-L1 inhibitors markedly improves OS and progression-free survival (PFS) in patients with small cell lung cancer (SCLC) (17-19). However, the suitability of immunotherapy for ENEC remains uncertain.

Emerging evidence suggests that the BRAF V600E mutation may contribute to immune evasion by upregulating PD-L1 expression, a phenomenon observed in several tumor types, including melanoma, colorectal cancer and non-small cell lung cancer (20). If this mutation is also present in ENEC, it may influence tumor immunogenicity and modulate the response to immunotherapy.

In the present study, a comprehensive analysis of the clinical characteristics and histopathological features of ENEC was performed. For the first time, the expression status of both BRAF V600E mutation and PD-L1 were simultaneously assessed in this rare carcinoma, contributing novel insights into the molecular landscape of ENEC. Additionally, potential clinical and pathological factors associated with patient prognosis were investigated, aiming to provide a theoretical and practical foundation for future therapeutic strategies.

Materials and methods

Patients. In the present study, clinical data from 3,816 patients with esophageal cancer who were admitted and treated at the Department of Thoracic Surgery, Affiliated Hospital of North Sichuan Medical College (Nanchong, China) between January 2019 and September 2024 were reviewed. The study was reviewed and approved by The Ethics Committee of Affiliated Hospital of North Sichuan Medical College (approval no. 2024ER682-1).

The patient selection process is illustrated in Fig. 1. A total of 66 patients with a pathological diagnosis of ENEC were identified based on the diagnostic criteria outlined in the 2019 World Health Organization Classification of Tumors of the Digestive System (21). Among these, 6 patients were initially suspected of having neuroendocrine carcinoma based on biopsy findings but were later confirmed to have different pathological types after surgical resection: A total of 5 cases were diagnosed with squamous cell carcinoma and 1 case with melanoma and 1 patient was found to have synchronous primary tumors, with squamous cell carcinoma in the middle esophagus and neuroendocrine carcinoma at the esophagogastric junction. Another patient had previously been diagnosed with esophageal squamous cell carcinoma and received definitive radiotherapy. Subsequently, 3 years later, post-surgical pathology revealed a mixed-type ENEC consisting of 30% squamous cell carcinoma and 70% large-cell neuroendocrine carcinoma components. Additionally, 2 patients with confirmed ENEC refused treatment. After excluding the aforementioned cases, a total of 56 patients were included in the final analysis, of whom 39 underwent surgical treatment and 17 received non-surgical management.

Follow-up and definitions. Patient follow-up data were collected through regular outpatient visits and telephone interviews, including information on tumor progression and survival status. All patients were followed up at 3-month intervals for a minimum of 6 months from the initiation of treatment. PFS was defined as the time from the start of treatment to the first confirmed disease progression or mortality from any cause. OS was defined as the time from the start of treatment to mortality from any cause. R0 resection was defined as a complete tumor resection with no microscopic residual tumor at the surgical margins.

Immunohistochemical staining. A total of 39 patients who underwent surgical resection and were confirmed to have ENEC based on postoperative pathological diagnosis were included in the immunohistochemical analysis with PD-L1 and BRAF V600E. Postoperative tumor specimens were retrieved from the Pathology Specimen Bank of the Affiliated Hospital of North Sichuan Medical College. The primary antibodies were anti-PD-L1 (cat. no. #13684; 1:100; Cell Signaling Technology, Inc.) and anti-BRAF V600E (cat. no. ab228461; 1:100; Abcam). Antibodies used for immunohistochemical identification of neuroendocrine differentiation and proliferative activity were as follows: Cytokeratin (CK; cat. no. HA721455; 1:200), chromogranin A (CgA; cat. no. ET1703-08; 1:1,000), synaptophysin (Syn; cat. no. HA723196; 1:200), CD56 (cat. no. HA722755; 1:1,000) and Ki-67 (cat. no. HA721115; 1:5,000; all Hangzhou Huaan Biotechnology Co., Ltd.).

Immunohistochemical staining was performed using the UltraSensitive™ S-P IHC kit (mouse/rabbit; Fuzhou Maixin Biotechnology Development Co., Ltd.) according to the manufacturer's instructions. Briefly, specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 μm. Primary antibody incubation was performed at room temperature, followed by secondary antibody and detection reagent application using the UltraSensitive™ S-P system with DAB as the chromogen. The secondary antibodies were included in the detection kit. Microscopic examination and image acquisition were performed using a light microscope with Olympus cellSens Entry software (version 4.2; Olympus Corporation).Human tonsil tissue was used as the positive control for PD-L1 staining, while thyroid tissue known to harbor the BRAF V600E mutation served as the positive control for BRAF V600E staining. For negative controls, phosphate buffered saline was substituted for the primary antibodies.

All slides were independently evaluated by two experienced pathologists with intermediate or senior professional titles. In cases of discordant interpretation, a joint review was performed, and final decisions were made by consensus. PD-L1 expression was evaluated based on membranous staining of tumor cells. Following criteria established in previous studies (22,23), a tumor was considered PD-L1 positive when ≥1% of tumor cells (tumor proportion score, TPS) demonstrated membranous staining with an intensity clearly above background levels. BRAF V600E expression was assessed based on cytoplasmic staining. Tumor cells were considered positive if distinct cytoplasmic staining was observed.

Statistical analysis. All statistical analyses were performed using IBM SPSS software (IBM Corp.; version 27.0). Continuous variables with normal distribution are expressed

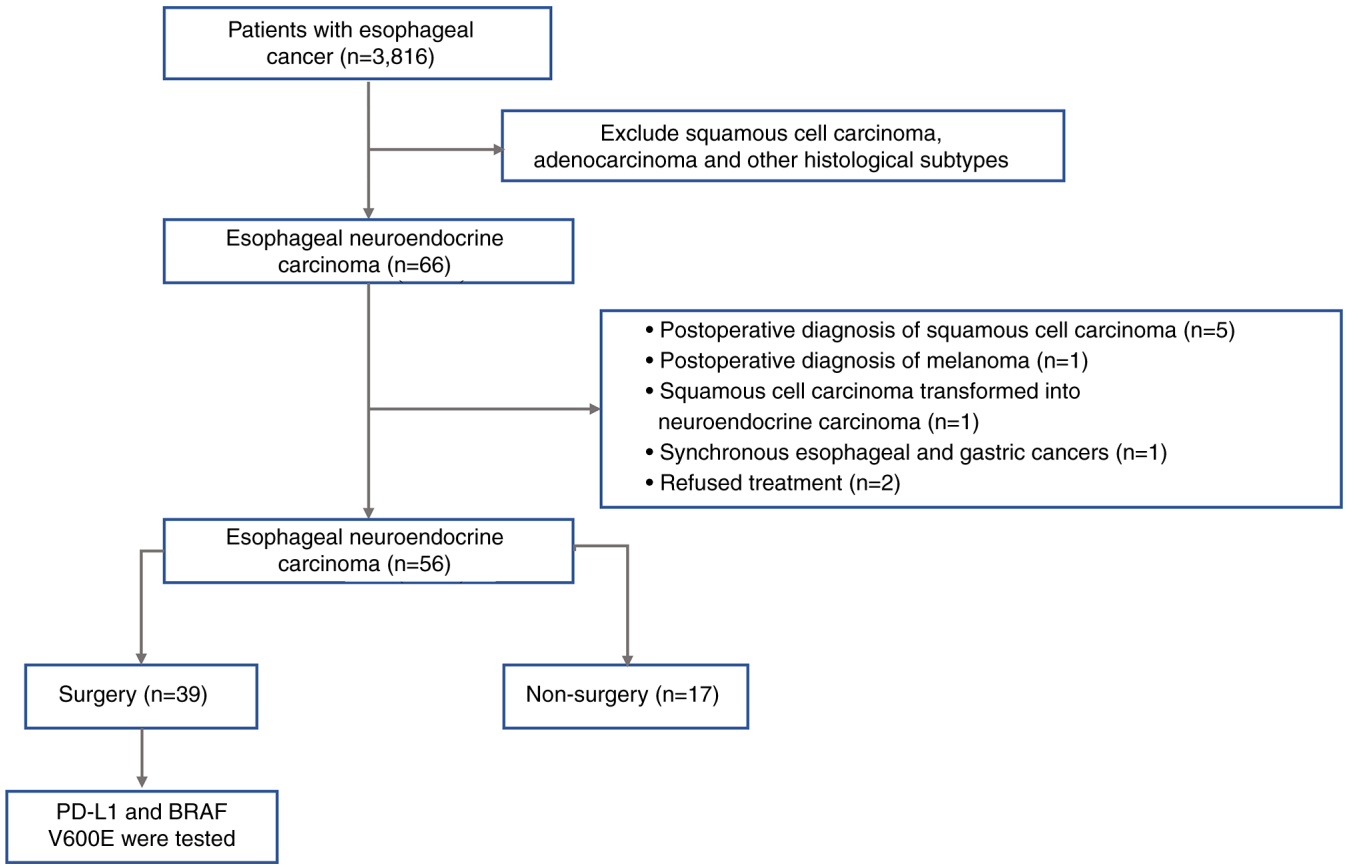

Figure 1. Flowchart of the present study.

as mean ± standard deviation and compared between groups using an independent samples t-test. Categorical variables were presented as frequency and percentage, and differences between groups were analyzed using a χ^2 test or Fisher's exact test, as appropriate. For ordinal categorical variables and continuous variables not conforming to normal distribution, non-parametric comparisons were performed using a rank sum test.

Survival outcomes, including PFS and OS, were estimated using the Kaplan-Meier method with corresponding 95% confidence intervals (CI). Differences in survival between groups were assessed using the log-rank test. Kaplan-Meier survival curves were generated using MedCalc software (MedCalc Software Ltd.; version 22.018). To further identify independent prognostic factors in patients with ENEC, univariate and multivariate Cox proportional hazards regression models were constructed. All statistical tests were two-tailed, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baseline characteristics. A total of 56 patients were included in the analysis. Baseline characteristics are summarized in Table I. The mean age of the patients was 67.1±8.3 years. The majority of patients were male (69.6%). Tumors were predominantly located in the middle (50.0%) and lower (41.1%) esophagus. The most common presenting symptom was dysphagia (60.7%), followed by subxiphoid pain (21.4%). In most cases (69.6%), the disease duration ranged from 1 to 6 months at the time of diagnosis.

Based on upper gastrointestinal imaging and contrast-enhanced chest computed tomography, tumor length was stratified into >3 and ≤3 cm, while tumor thickness was categorized as >1 and ≤1 cm. Among all patients, 73.2% had tumors >3 cm in length, and 58.9% had tumors >1 cm in thickness. Clinical staging revealed that 60.7% of patients were at tumor (T)3/T4 stage, and 46.4% were at lymph node (N)1-N3 stage. Distant metastasis was observed in 2 patients (3.6%) at initial diagnosis.

Patients were divided into surgical and non-surgical groups based on treatment strategy. Significant differences were observed between the two groups in tumor length and thickness and clinical T and clinical N stage. No statistically significant differences were noted for other baseline characteristics, and the groups were otherwise comparable (Table I).

Surgical data. In the present study cohort, a total of 39 patients received surgery. As shown in Table SI, the R0 resection rate was 97.4% (n=38), and minimally invasive surgery was performed in 79.5% (n=31) of cases. The number of lymph nodes that were intraoperatively excised was 15 (range, 4-50). The mean operative time was 210 min (range, 133-365 min), and the mean intraoperative blood loss was 80 ml (range, 10-500 ml). The median postoperative hospital stay was 10 days (range, 7-42 days). Postoperative complications were most common with pulmonary infection with an incidence of 28.2% (n=11), followed by anastomotic fistula (5.1%, n=2). A total of 79.4%

Table I. Patient characteristics.

Characteristic	Total (n=56)	Non-surgery (n=17)	Surgery (n=39)	P-value
Age, mean ± SD	67.1±8.3	67.4±7.8	67.0±8.6	0.893
BMI, mean ± SD	22.3±3.3	21.5±2.4	22.7±3.5	0.197
Sex				0.463
Female	17 (30.4)	4 (23.5)	13 (33.3)	
Male	39 (69.6)	13 (76.5)	26 (66.7)	
Smoking				0.270
No	26 (46.4)	6 (35.3)	20 (51.3)	
Yes	30 (53.6)	11 (64.7)	19 (48.7)	
Drinking				0.075
No	33 (58.9)	7 (41.2)	26 (66.7)	
Yes	23 (41.1)	10 (58.8)	13 (33.3)	
Hypertension				0.800
No	44 (78.6)	13 (76.5)	31 (79.5)	
Yes	12 (21.4)	4 (23.5)	8 (20.5)	
Diabetes				0.908
No	53 (94.6)	16 (94.1)	37 (94.9)	
Yes	3 (5.4)	1 (5.9)	2 (5.1)	
Tumor location				0.302
Upper	3 (5.4)	1 (5.9)	2 (5.1)	
Middle	28 (50.0)	11 (64.7)	17 (43.6)	
Lower	23 (41.1)	5 (29.4)	20 (51.3)	
Length of tumor				0.023
≤3 cm	15 (26.8)	1 (5.9)	14 (35.9)	
>3 cm	41 (73.2)	16 (94.1)	25 (64.1)	
Thickness of tumor				0.003
≤10 mm	23 (41.1)	2 (11.8)	21 (53.8)	
>10 mm	33 (58.9)	15 (88.2)	18 (46.2)	
Symptom				0.179
Dysphagia	34 (60.7)	12 (70.6)	22 (56.4)	
Subxiphoid pain	12 (21.4)	1 (5.9)	11 (28.2)	
Dysphagia with pain	6 (10.7)	3 (17.6)	3 (7.7)	
Medical screening	4 (7.1)	1 (5.9)	3 (7.7)	
Disease duration				0.308
<1 month	14 (25.0)	4 (23.5)	10 (25.6)	
1-6 months	39 (69.6)	13 (76.5)	26 (66.7)	
>6 months	3 (5.4)	0 (0.0)	3 (7.7)	
Clinical T stage				0.005
T1/T2	22 (39.3)	2 (11.8)	20 (51.3)	
T3/T4	34 (60.7)	15 (88.2)	19 (48.7)	
Clinical N stage				<0.001
N0	30 (53.6)	1 (5.9)	29 (74.4)	
N+	26 (46.4)	16 (94.1)	10 (25.6)	
Clinical M stage				0.088
M0	54 (96.4)	15 (88.2)	39 (100.0)	
M1	2 (3.6)	2 (11.8)	0 (0.0)	

Data presented as n (%). T, tumor; N, lymph node; M, metastasis.

(n=31) of patients did not receive preoperative neoadjuvant therapy and 25 (64.1%) did not receive postoperative adjuvant therapy.

As presented in Table II, postoperative pathological analysis revealed that the most common macroscopic tumor type was ulcer type (53.8%; n=21), while the narrowing type was the

Table II. Pathological results.

Pathological result	Surgical group (n=39)
Morphological subtype	
Mushroom type	6 (15.4)
Ulcer type	21 (53.8)
Medullary type	6 (15.4)
Narrowing type	1 (2.6)
Mucosal abnormality	5 (12.8)
CK	
(-)	4 (10.3)
(+)	20 (51.3)
Absent	15 (38.5)
CgA	
(-)	23 (59.0)
(+)	13 (33.3)
Absent	3 (7.7)
Syn	
(-)	4 (10.3)
(+)	35 (89.7)
CD56	
(-)	6 (15.4)
(+)	33 (84.6)
Ki-67, %	
≤55	10 (25.6)
>55	29 (74.4)
PD-L1 (+)	6 (15.4)
BRAF V600E (+)	5 (12.8)
Pathological T stage	
T1a	2 (5.1)
T1b	13 (33.3)
T2	10 (25.6)
T3	14 (35.9)
Pathological N stage	
N0	18 (46.2)
N1	11 (28.2)
N2	7 (17.9)
N3	3 (7.7)
Vascular invasion	8 (20.5)
Perineural invasion	3 (7.7)
Preoperative diagnosis	
Squamous cell carcinoma	13 (33.3)
Neuroendocrine carcinoma	15 (38.5)
Diagnosis uncertain	11 (28.2)
Postoperative diagnosis	
Small cell neuroendocrine carcinoma	28 (71.7)
Large cell neuroendocrine carcinoma	1 (2.6)
MiNEC-SCC	9 (23.1)
MiNEC-AC	1 (2.6)

Data presented as n (%). (+), positive; (-), negative; MiNEC-SCC, mixed neuroendocrine carcinoma and squamous cell carcinoma; MiNEC-AC, mixed neuroendocrine carcinoma and adenocarcinoma; CK, cytokeratin; CgA, chromogranin A, Syn, synaptophysin; PD-L1, programmed death ligand 1; T, tumor; N, lymph node.

least frequent (2.6%; n=1; Table II). Immunohistochemical staining showed positive expression of cytokeratin in 51.3% of cases, chromogranin A in 33.3%, synaptophysin in 89.7% and CD56 in 84.6%. A high proliferative index, defined as Ki-67 >55%, was observed in 74.4% of patients. The incidence of perineural invasion was 7.7%, and vascular invasion was identified in 20.5% of cases.

According to the pathological T, N, metastasis (M) staging, 64.1% of patients were classified as T1-T2, while 35.9% were staged as T3. Lymph node metastasis was observed in 53.8% of patients. A, 74.3% of tumors were classified as pure neuroendocrine carcinoma, with small cell neuroendocrine carcinoma (SCNEC) being the predominant subtype (71.7%). Additionally, 23.1% of tumors were identified as mixed neuroendocrine carcinoma and squamous cell carcinoma (MiNEC-SCC), and 2.6% were classified as mixed neuroendocrine carcinoma and adenocarcinoma.

Expression and clinical characterization of PD-L1 and BRAF V600E. Immunohistochemical staining for PD-L1 and BRAF V600E was performed in patients who received surgery to evaluate their expression profiles in ENEC and to explore the clinical characteristics associated with biomarker positivity. As shown in Fig. S1, PD-L1 was normally expressed on the cell membrane, while BRAF V600E is normally expressed in the cytoplasm (Fig. S1). As shown in Table II, the BRAF V600E mutation was detected in 12.8% of cases (5/39), while PD-L1 positivity was observed in 15.4% (6/39).

To further investigate potential clinical associations, patients were stratified into biomarker-positive and -negative groups. As shown in Table SII, a significant difference in presenting symptoms was identified between the PD-L1 positive and negative groups. In the PD-L1 positive group, subxiphoid pain was the most common initial symptom (50%), and 83.3% of patients reported pain-related symptoms. By contrast, the PD-L1 negative group predominantly presented with dysphagia (63.6%). Tumor length also differed significantly between the BRAF V600E positive and negative groups. In the positive group, 80% of tumors were ≤3 cm in length, whereas 70.6% of tumors in the negative group measured >3 cm.

As summarized in Table SIII, a pooled analysis of patients with positive PD-L1 and BRAF expression was performed. Among the 9 patients with positive biomarker expression, 88.9% (8/9) were >60 years of age. The most common pathological subtype in this group was MiNEC-SCC, accounting for 50.0% (3/6) of PD-L1-positive cases and 60.0% (3/5) of BRAF V600E-positive cases. This was followed by SCNEC, observed in 33.3% (2/6) of PD-L1-positive cases and 40.0% (2/5) of BRAF V600E-positive cases. Notably, two patients with MiNEC-SCC were found to co-express both PD-L1 and BRAF V600E.

Survival outcomes. As illustrated in Fig. 2, the median follow-up duration for the entire cohort was 16.8 months. In the overall population, the median OS was 28.3 months and the median PFS was 10.6 months. The 1-year OS and PFS rates were 79.7 and 43.1%, respectively, while the 3-year OS and PFS rates were 49.5 and 25.1%, respectively.

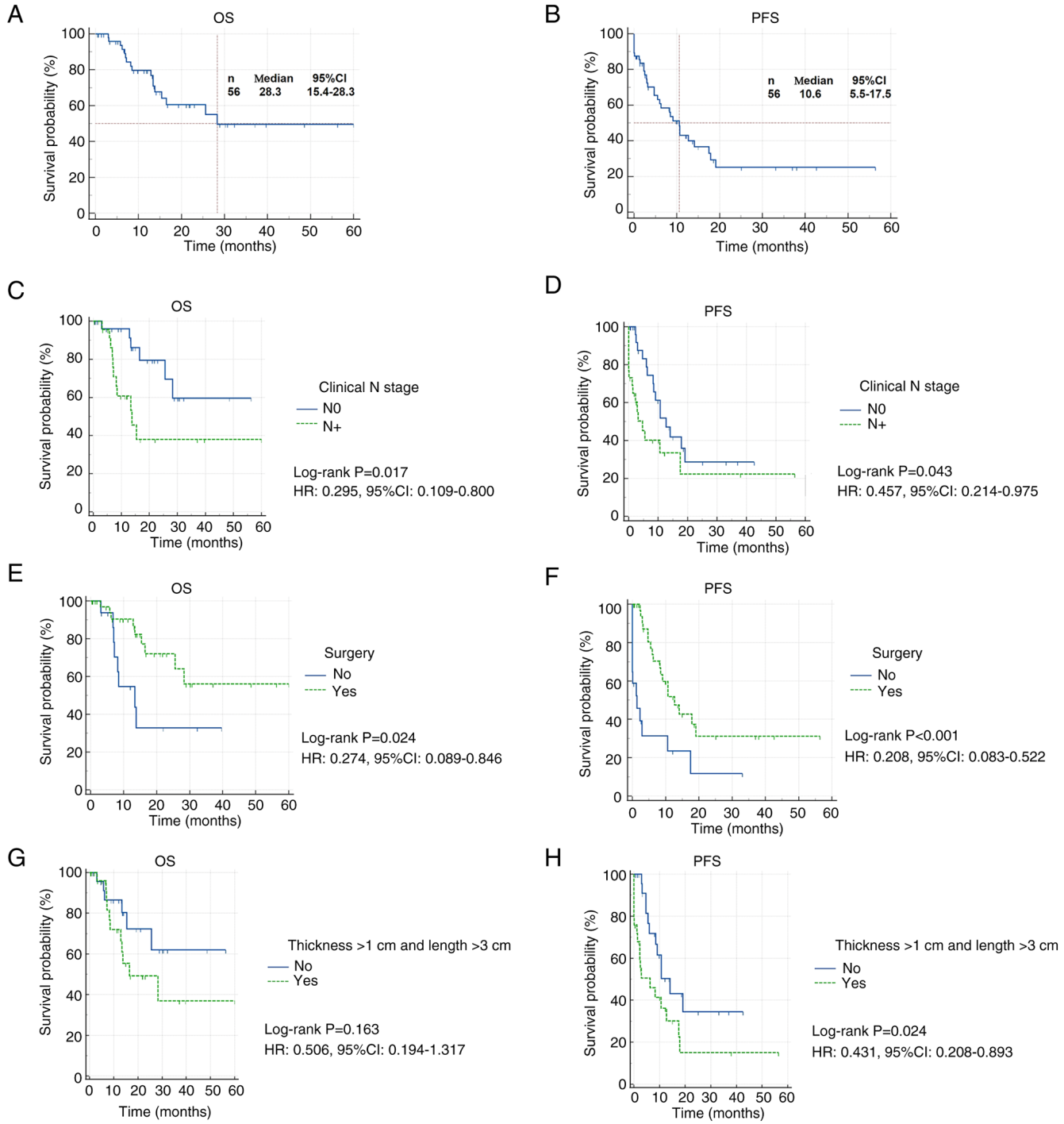

Figure 2. OS and PFS of the entire cohort. (A) OS of the entire cohort. (B) PFS of the entire cohort. (C) OS of clinical N0 and N+ groups. (D) PFS of clinical N0 and N+ groups. (E) OS of surgery and non-surgery groups. (F) PFS of surgery and non-surgery groups. (G) OS of more aggressive and less aggressive groups. (H) PFS of more aggressive and less aggressive groups. PFS, progression-free survival; OS, overall survival; N, node; HR, hazard ratio; CI, confidence interval.

Survival analysis by clinical N stage showed that patients with N0 disease had significantly improved outcomes compared with those with N1-N3 disease. Specifically, N0 patients exhibited longer OS [hazard ratio (HR), 0.295, 95% CI, 0.109-0.800] and PFS (HR, 0.450; 95% CI, 0.214-0.975). Patients who received surgery demonstrated significantly improved survival compared with those who did not. OS was significantly longer in the surgical group (HR, 0.274; 95% CI, 0.089-0.846) and PFS was also significantly prolonged (HR, 0.208; 95% CI, 0.083-0.522). Tumors with a thickness >1 cm and length >3 cm were defined as more aggressive. Patients with less aggressive tumors showed improved PFS compared with those with more aggressive disease (HR, 0.431; 95% CI, 0.208-0.893), while no statistically significant difference in OS was observed.

As shown in Table III, univariate Cox regression analysis identified clinical N stage and surgery as prognostic factors for OS; however, these were not retained as independent predictors in the multivariate model. For PFS, univariate analysis revealed that clinical N stage, tumor aggressiveness and surgery were

Table III. Univariate and multivariate Cox regression analysis in the overall population.

	OS				PFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Characteristic	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Clinical N stage								
N0								
N1-3	3.208 (1.173, 8.775)	0.023	2.469 (0.619, 9.853)	0.200	2.040 (0.999, 4.163)	0.050	0.916 (0.344, 2.435)	0.860
Tumor size and length								
Thickness ≤1 cm or length ≤3 cm								
Thickness >1 cm and length >3 cm	2.005 (0.739, 5.441)	0.172	0.958 (0.265, 3.462)	0.948	2.244 (1.084, 4.648)	0.030	1.956 (0.816, 4.689)	0.133
Surgical treatment								
No								
Yes	0.346 (0.132, 0.910)	0.031	0.557 (0.178, 1.743)	0.315	0.314 (0.152, 0.650)	0.002	0.345 (0.146, 0.816)	0.015

N, lymph node; CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

significantly associated with prognosis. Multivariate analysis demonstrated that surgery was an independent predictor of improved PFS (HR, 0.345; 95% CI, 0.146-0.816).

In the surgical cohort, survival analysis was performed with a median follow-up duration of 15 months. As shown in Fig. 3, the median OS was not reached, while the median PFS was 12.7 months. The 1-year OS and PFS rates were 93.6 and 51.8%, respectively, while the 3-year OS and PFS rates were 58.1 and 31.2%, respectively.

Patients who received postoperative adjuvant therapy demonstrated significantly improved survival outcomes compared with those who did not. Specifically, adjuvant therapy was associated with prolonged OS (HR, 0.233; 95% CI, 0.057-0.952) and improved PFS (HR, 0.316; 95% CI, 0.121-0.822). As shown in Table SIV, Cox regression analysis further demonstrated that postoperative adjuvant therapy was an independent prognostic factor for PFS in the surgical cohort (HR, 0.252; 95% CI,0.081-0.781).

Discussion

ENEC most commonly exhibits small cell morphology, resembling SCLC, rather than the large-cell morphology typically seen in other gastrointestinal neuroendocrine carcinomas (GINECs). In the present study, 71.7% (28/39) of patients had tumors classified as SCNEC. Molecular analyses have revealed notable overlap between ENEC and SCLC in terms of genomic alterations and molecular subtypes (24,25). Due to the lack of disease-specific treatment guidelines, ENEC is currently managed using protocols extrapolated from either SCLC or other gastrointestinal neuroendocrine tumors.

PD-L1 expression has been reported in >70% of SCLC cases and is considered a favorable prognostic biomarker (26). This success has prompted interest in the potential role of immunotherapy for ENEC. However, data on PD-L1 expression in ENEC are limited and inconsistent. Yamashita *et al* (27) reported a PD-L1 positivity rate of 33% in a small ENEC cohort. In another study, Huang *et al* (28) similarly found a PD-L1 positivity rate of 33% (3/9). By contrast, Xing *et al* (29) reported a notably lower PD-L1 positivity rate of 9.1% in ENEC, compared with 29% in GINEC, raising concerns about the applicability of PD-1/PD-L1 monotherapy in ENEC. These discrepancies highlight the potential need for combination therapy strategies in this patient population.

The BRAF V600E mutation may upregulate PD-L1 expression, thereby promoting immune evasion (20). Preclinical studies have shown that combining immune checkpoint inhibitors with BRAF-targeted therapy may enhance treatment efficacy in BRAF-mutated tumors (30,31). Tian *et al* (20) reported that the combination of PD-1, BRAF and MEK inhibitors results in a notably higher objective response rate (25.0%; 95% CI, 10.7-44.9%) compared with BRAF and MEK inhibition alone (7.0%; 95% CI, 1.5-19.1%), highlighting the potential synergistic benefit of incorporating immunotherapy into targeted therapy regimens.

In the present study, the expression profiles of PD-L1 and BRAF V600E mutations were analyzed in ENEC to explore potential targets for such combination strategies. The results revealed a relatively low prevalence of BRAF V600E mutations (12.8%; 5/39) and PD-L1 positivity (15.4%; 6/39), suggesting that ENEC may lack the features of a highly immune-infiltrated tumor microenvironment and instead represents an 'immune cold' phenotype distinct from SCLC. Consistent with the present findings, Zhang *et al* (32) previously demonstrated that ENEC tumors exhibited sparse immune cell infiltration, which may contribute to reduced antitumor immune responses and

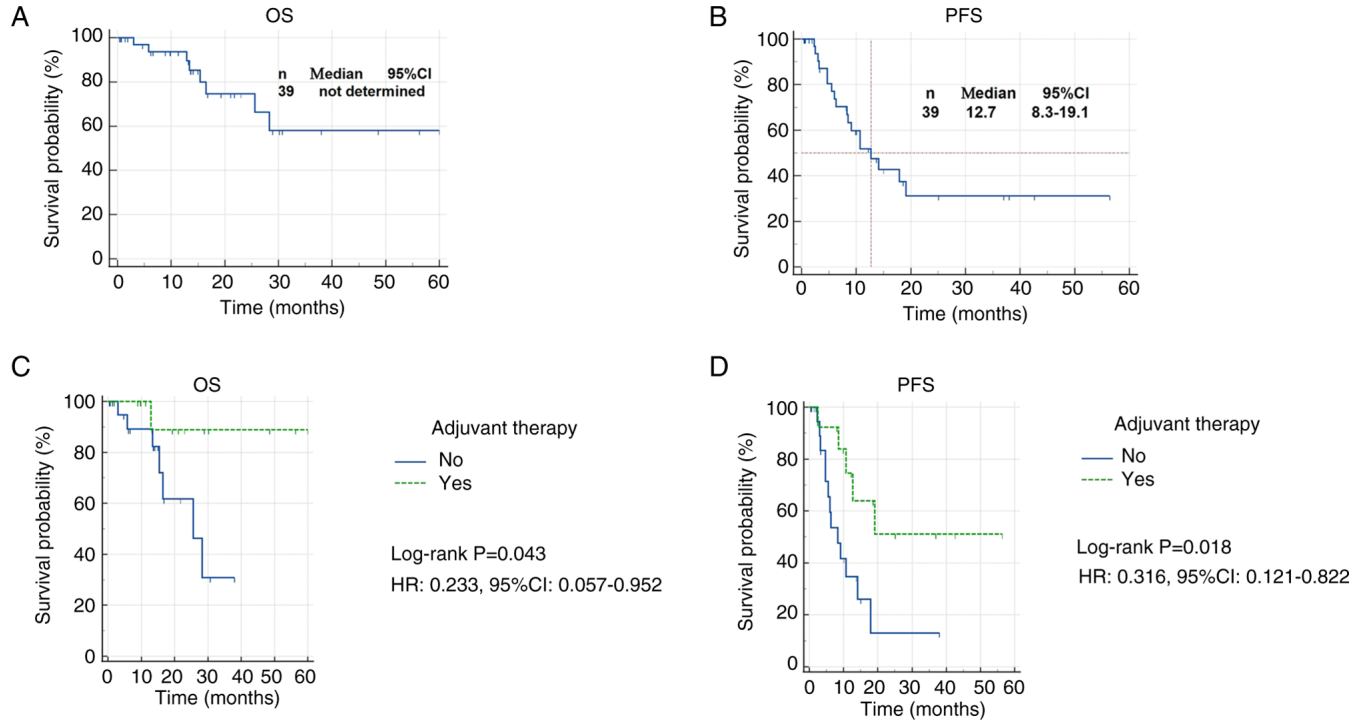

Figure 3. OS and PFS of the surgical cohort. (A) OS of the surgical cohort. (B) PFS of the surgical cohort. (C) OS of patients with adjuvant therapy and without adjuvant therapy groups. (D) PFS of patients with adjuvant therapy and without adjuvant therapy groups. PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

limited immunotherapeutic efficacy. However, the expression of PD-L1 in ENEC needs to be further clarified in studies with larger samples.

Furthermore, the present results corroborate previous studies showing that the BRAF mutation rate in ENEC is markedly lower compared with in other GINECs (9). These observations imply that BRAF V600E is unlikely to be a principal oncogenic driver in ENEC, and its clinical phenotype may not be associated with mutation status. The present study observed that clinical symptoms differed between the PD-L1 positive and negative groups, while tumor length showed a significant difference between the BRAF V600E-positive and negative groups. However, due to the limited number of positive cases, potential bias cannot be ruled out, and these associations should be interpreted with caution.

Notably, however, PD-L1 and BRAF V600E expression was notably more frequent in MiNEC-SCC subtypes, with a positivity rate of 33.3% (3/9) for both markers. This raises the possibility that BRAF V600E mutation and PD-L1 expression may be involved in the phenotypic plasticity or lineage conversion between neuroendocrine and squamous histology. Therefore, screening for relevant markers in patients with MiNEC-SCC is recommended, who are more likely to benefit from immunotherapy or targeted therapy.

ENEC is characterized by its high aggressiveness and early propensity for metastasis (33). In the present cohort, two patients presented with distant metastases at initial diagnosis, and 46.4% were clinically staged as N1 or higher. Among the surgical population, 74.4% of tumors exhibited a Ki-67 proliferation index >55%, indicating a high proliferative potential. The incidences of perineural invasion and vascular invasion were 7.7 and 20.5%, respectively. Although 64.1% of patients were pathologically staged as T1 or T2, the lymph node positivity rate reached 53.8%, suggesting a strong tendency for early lymphatic spread and vascular invasion even in tumors classified as early-stage.

Patients with ENEC have an extremely poor prognosis (34,35). A large systematic review including 1,176 patients with esophageal SCNEC reported a median OS of only 11.1 months (36). In the present study cohort, the median OS reached 28.3 months, and the median PFS was 10.6 months. The 1-year OS and PFS rates were 79.7 and 43.1%, respectively, while the 3-year OS and PFS rates were 49.5 and 25.1%. Subgroup analysis revealed that advanced N stage and more aggressive tumor characteristics (thickness >1 cm and length >3 cm) were associated with worse PFS outcomes, consistent with the findings of Zou *et al* (37). Surgery significantly improved survival outcomes. Patients who underwent surgery exhibited longer OS and PFS compared with those who did not receive surgery. Moreover, multivariate Cox regression analysis identified surgery as an independent prognostic factor for PFS. Although potential selection bias cannot be excluded-due to certain patients being ineligible for surgery due to advanced disease-the survival advantage associated with surgical intervention remains evident. Similar findings have been reported in previous studies. Erdem *et al* (38) found that the 2-year OS was significantly higher in the surgical group (57.3%) compared with the non-surgical group (35.2%). Xu *et al* (4) demonstrated that patients with stage I or IIA esophageal SCNEC who underwent surgery alone had improved survival compared with those who did not (median OS, 29.0 vs. 17.4 months; P=0.031). Chen *et al* (34) also reported a survival benefit associated with surgery (P=0.003).

In the present surgical cohort, the median PFS was 12.7 months. The 1-year OS and PFS rates were 93.6 and 51.8%, respectively, while the 3-year OS and PFS were 58.1 and 31.2%. Although limited by a relatively short follow-up period and small sample size, the modest 1-year PFS highlights the aggressive nature of ENEC and its tendency toward early progression. Postoperative adjuvant therapy emerged as another key factor influencing prognosis. Patients who received adjuvant therapy after surgery had a significantly reduced risk of disease progression, by nearly 70%. Although the OS benefit was not statistically significant in multivariate Cox analysis, Kaplan-Meier curves revealed a trend toward prolonged OS in the adjuvant therapy group. Several previous studies have demonstrated that surgery combined with adjuvant therapy markedly improves outcomes in ENEC (37,39-41). However, some reports suggest that definitive chemoradiotherapy may provide superior survival benefits in patients with esophageal SCNEC compared with surgery combined with chemotherapy (42). Zhu *et al* (43) further indicated that patients with tumors located in the lower third of the esophagus or those with tumors >5 cm derived greater OS benefit from surgery plus chemotherapy, while chemoradiotherapy was more effective in tumors located in the middle third or ≤5 cm in length. More large-sample studies are needed in the future to determine the best treatment for patients with ENEC.

A notable limitation of the present study is the relatively small sample size, with only 39 patients available for analysis. No formal power analysis was conducted, as the rarity of ENEC inherently restricted case accrual, which may have limited the statistical power. In addition, immunohistochemical images for CK, Syn, CgA, and Ki-67 were not available, as these data were retrieved from electronic medical records rather than original pathological slides. Nevertheless, to the best of our knowledge, this represents one of the largest single-institution cohorts to date that systematically evaluated PD-L1 expression and BRAF V600E mutation in ENEC. While the findings should be interpreted with caution, they provide valuable preliminary evidence and may serve as a reference point for future multi-center studies with larger cohorts to validate these results.

In conclusion, PD-L1 expression and BRAF V600E mutations are rare in ENEC but more common in MiNEC-SCC. Due to the overall poor prognosis of ENEC, surgery combined with adjuvant therapy may offer clinical benefit for selected patients.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MF performed the study design, CZ performed the statistical analysis and was a major contributor in writing the manuscript. LZ and BX performed experiments. GS interpreted data. CZ and MF confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was reviewed and approved by The Ethics Committee of Affiliated Hospital of North Sichuan Medical College (approval no. 2024ER682-1). According to national legislation and institutional requirements, written informed consent was not required for participation in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Li Z, Hu J, Chen P and Zeng Z: Incidence, treatment, and survival analysis in esophageal neuroendocrine carcinoma population. Transl Cancer Res 9: 4317-4329, 2020.
2. Wong AT, Shao M, Rineer J, Osborn V, Schwartz D and Schreiber D: Treatment and survival outcomes of small cell carcinoma of the esophagus: An analysis of the National Cancer Data Base. Dis Esophagus 30: 1-5, 2017.
3. Ji A, Jin R, Zhang R and Li H: Primary small cell carcinoma of the esophagus: Progression in the last decade. Ann Transl Med 8: 502, 2020.
4. Xu L, Li Y, Liu X, Sun H, Zhang R, Zhang J, Zheng Y, Wang Z, Liu S and Chen X: Treatment strategies and prognostic factors of Limited-stage primary small cell carcinoma of the esophagus. J Thorac Oncol 12: 1834-1844, 2017.
5. Roskoski R: Targeting oncogenic Raf protein-serine/threonine kinases in human cancers. Pharmacol Res 135: 239-258, 2018.
6. Roskoski R: RAF protein-serine/threonine kinases: Structure and regulation. Biochem Biophys Res Commun 399: 313-317, 2010.
7. Beeram M, Patnaik A and Rowinsky EK: Raf: A strategic target for therapeutic development against cancer. J Clin Oncol 23: 6771-6790, 2005.
8. Fazio N, Abdel-Rahman O, Spada F, Galdy S, De Dosso S, Capdevila J and Scarpa A: RAF signaling in neuroendocrine neoplasms: From bench to bedside. Cancer Treat Rev 40: 974-979, 2014.
9. Dizdar L, Werner TA, Drusenheimer JC, Möhlendick B, Raba K, Boeck I, Anlauf M, Schott M, Göring W, Esposito I, *et al*: BRAFV600E mutation: A promising target in colorectal neuroendocrine carcinoma. Int J Cancer 144: 1379-1390, 2019.
10. Idrees K, Padmanabhan C, Liu E, Guo Y, Gonzalez RS, Berlin J, Dahlman KB, Beauchamp RD and Shi C: Frequent BRAF mutations suggest a novel oncogenic driver in colonic neuroendocrine carcinoma. J Surg Oncol 117: 284-289, 2018.
11. Capdevila J, Arqués O, Hernández Mora JR, Matito J, Caratù G, Mancuso FM, Landolfi S, Barriuso J, Jimenez-Fonseca P, Lopez Lopez C, *et al*: Epigenetic EGFR gene repression confers sensitivity to therapeutic BRAFV600E blockade in colon neuroendocrine carcinomas. Clin Cancer Res 26: 902-909, 2020.
12. Pedoeem A, Azoulay-Alfaguter I, Strazza M, Silverman GJ and Mor A: Programmed death-1 pathway in cancer and autoimmunity. Clin Immunol 153: 145-152, 2014.

13. Chen X, Xu X, Wang D, Liu J, Sun J, Lu M, Wang R, Hui B, Li X, Zhou C, *et al*: Neoadjuvant sintilimab and chemotherapy in patients with potentially resectable esophageal squamous cell carcinoma (KEEP-G 03): An Open-label, single-arm, phase 2 trial. J Immunother Cancer 11: e005830, 2023.
14. Liu J, Yang Y, Liu Z, Fu X, Cai X, Li H, Zhu L, Shen Y, Zhang H, Sun Y, *et al*: Original research: Multicenter, single-arm, phase II trial of camrelizumab and chemotherapy as neoadjuvant treatment for locally advanced esophageal squamous cell carcinoma. J Immunother Cancer 10: e004291, 2022.
15. Yan X, Duan H, Ni Y, Zhou Y, Wang X, Qi H, Gong L, Liu H, Tian F, Lu Q, *et al*: Tislelizumab combined with chemotherapy as neoadjuvant therapy for surgically resectable esophageal cancer: A prospective, single-arm, phase II study (TD-NICE). Int J Surg 103: 106680, 2022.
16. Sun JM, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, Kojima T, Metges JP, Li Z, Kim SB, *et al*: Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): A randomised, placebo-controlled, phase 3 study. Lancet 398: 759-771, 2021.
17. Paz-Ares L, Dvorkin M, Chen Y, Reinmuth N, Hotta K, Trukhin D, Statsenko G, Hochmair MJ, Özgüroğlu M, Ji JH, *et al*: Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): A randomised, controlled, open-label, phase 3 trial. Lancet 394: 1929-1939, 2019.
18. Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, Huemer F, Losonczy G, Johnson ML, Nishio M, *et al*: First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 379: 2220-2229, 2018.
19. Cheng Y, Han L, Wu L, Chen J, Sun H, Wen G, Ji Y, Dvorkin M, Shi J, Pan Z, *et al*: Effect of First-line serplulimab vs placebo added to chemotherapy on survival in patients with Extensive-stage small cell lung cancer: The ASTRUM-005 randomized clinical trial. JAMA 328: 1223-1232, 2022.
20. Tian J, Chen JH, Chao SX, Pelka K, Giannakis M, Hess J, Burke K, Jorgji V, Sindurakar P, Braverman J, *et al*: Combined PD-1, BRAF and MEK inhibition in BRAFV600E colorectal cancer: A phase 2 trial. Nat Med 29: 458-466, 2023.
21. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F and Cree IA; WHO Classification of Tumours Editorial Board: The 2019 WHO classification of tumours of the digestive system. Histopathology 76: 182-188, 2020.
22. Guleria P, Kumar S, Malik PS and Jain D: PD-L1 expression in small cell and large cell neuroendocrine carcinomas of lung: An immunohistochemical study with review of literature. Pathol Oncol Res 26: 2363-2370, 2020.
23. Lantuejoul S, Sound-Tsao M, Cooper WA, Girard N, Hirsch FR, Roden AC, Lopez-Rios F, Jain D, Chou TY, Motoi N, *et al*: PD-L1 testing for lung cancer in 2019: Perspective from the IASLC pathology committee. J Thorac Oncol 15: 499-519, 2020.
24. Li R, Yang Z, Shao F, Cheng H, Wen Y, Sun S, Guo W, Li Z, Zhang F, Xue L, *et al*: Multi-omics profiling of primary small cell carcinoma of the esophagus reveals RB1 disruption and additional molecular subtypes. Nat Commun 12: 3785, 2021.
25. Ooki A, Osumi H, Fukuda K and Yamaguchi K: Potent molecular-targeted therapies for gastro-entero-pancreatic neuroendocrine carcinoma. Cancer Metastasis Rev 42: 1021-1054, 2023.
26. Ishii H, Azuma K, Kawahara A, Yamada K, Imamura Y, Tokito T, Kinoshita T, Kage M and Hoshino T: Significance of programmed cell Death-ligand 1 expression and its association with survival in patients with small cell lung cancer. J Thorac Oncol 10: 426-430, 2015.
27. Yamashita S, Abe H, Yamashita H, Yagi K, Seto Y and Ushiku T: PD-L1 and HLA-class I expression status and their therapeutic implication in oesophageal small-cell carcinoma. Histopathology 83: 264-275, 2023.
28. Huang Z, Jin Y, Cai X, Chen L, Shen X, Li B, Chen H and Li Y: Association of the programmed death ligand-1 combined positive score in tumors and clinicopathological features in esophageal cancer. Thorac Cancer 13: 523-532, 2022.
29. Xing J, Ying H, Li J, Gao Y, Sun Z, Li J, Bai C, Cheng Y and Wu H: Immune checkpoint markers in neuroendocrine carcinoma of the digestive system. Front Oncol 10: 132, 2020.
30. Ebert PJR, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, Gould SE, Maecker H, Irving BA, Kim JM, *et al*: MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity 44: 609-621, 2016.
31. Liu L, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, Yang J, Seestaller-Wehr L, Zhang SY, Hopson C, *et al*: The BRAF and MEK inhibitors dabrafenib and trametinib: Effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. Clin Cancer Res 21: 1639-1651, 2015.
32. Zhang L, Yu B, Liu Z, Wei J, Pan J, Jiang C and Li Z: Analysis of the clinicopathological characteristics, prognosis, and lymphocyte infiltration of esophageal neuroendocrine neoplasms: A Surgery-based cohort and Propensity-score matching study. Cancers (Basel) 15: 1732, 2023.
33. Giannetta E, Guarnotta V, Rota F, de Cicco F, Grillo F, Colao A and Faggiano A; NIKE: A rare rarity: Neuroendocrine tumor of the esophagus. Crit Rev Oncol Hematol 137: 92-107, 2019.
34. Chen C, Hu H, Zheng Z, Yang Y, Chen W, Qiao X, Li P and Zhang S: Clinical characteristics, prognostic factors, and survival trends in esophageal neuroendocrine carcinomas: A population-based study. Cancer Med 11: 4935-4945, 2022.
35. Gu YM, Yang YS, Shi GD, Yan CY, Shang QX, Zhang HL, Wang WP, Yuan Y and Chen LQ: Limited-stage small cell carcinoma of the esophagus treated with curative esophagectomy: A multicenter retrospective cohort study. J Surg Oncol 126: 1396-1402, 2022.
36. Lu X, Luo J, Ling Y, Kong YZ, Feng LL, Zhou J and Wang F: Management of small cell carcinoma of esophagus in China. J Gastrointest Surg 17: 1181-1187, 2013.
37. Zou B, Li T, Zhou Q, Ma D, Chen Y, Huang M, Peng F, Xu Y, Zhu J, Ding Z, *et al*: Adjuvant therapeutic modalities in primary small cell carcinoma of esophagus patients: A retrospective cohort study of multicenter clinical outcomes. Medicine (Baltimore) 95: e3507, 2016.
38. Erdem S, Troxler E, Warschkow R, Tsai C, Yerokun B, Schmied B, Stettler C, Blazer DG III, Hartwig M, Worni M and Gloor B: Is There a role for surgery in patients with neuroendocrine tumors of the esophagus? A Contemporary View from the NCDB. Ann Surg Oncol 27: 671-680, 2020.
39. Deng HY, Ni PZ, Wang YC, Wang WP and Chen LQ: Neuroendocrine carcinoma of the esophagus: Clinical characteristics and prognostic evaluation of 49 cases with surgical resection. J Thorac Dis 8: 1250-1256, 2016.
40. Zhou Y, Hou P, Zha KJ, Wang F, Zhou K, He W and Gao JB: Prognostic value of pretreatment contrast-enhanced computed tomography in esophageal neuroendocrine carcinoma: A multi-center follow-up study. World J Gastroenterol 26: 4680-4693, 2020.
41. Liu S, Ge X, Gao Z, Zhou Q, Shi Y, Jiang W, Yang M and Sun X: Clinicopathological analysis of 67 cases of esophageal neuroendocrine carcinoma and the effect of postoperative adjuvant therapy on prognosis. Medicine (Baltimore) 100: e27302, 2021.
42. Meng MB, Zaorsky NG, Jiang C, Tian LJ, Wang HH, Liu CL, Wang J, Tao Z, Sun Y, Wang J, *et al*: Radiotherapy and chemotherapy are associated with improved outcomes over surgery and chemotherapy in the management of limited-stage small cell esophageal carcinoma. Radiother Oncol 106: 317-322, 2013.
43. Zhu J, Wang Y, Sun H, Zhang Y, Zhang W, Shen W, Yang N, Tan B, Su X, Li L, *et al*: Surgery versus radiotherapy for limited-stage small cell esophageal carcinoma: A multicenter, retrospective, cohort study in China (ChiSCEC). Int J Surg 110: 956-964, 2024.