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Phase II study of temozolomide monotherapy in patients with extrapulmonary neuroendocrine carcinoma

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Abstract

Extrapulmonary neuroendocrine carcinoma (EPNEC) is a lethal disease with a poor prognosis. Platinum-based chemotherapy is used as the standard first-line treatment for unresectable EPNEC. Several retrospective studies have reported the results of the utilization of temozolomide (TMZ) as a drug for the second-line treatment for EPNEC. Patients with unresectable EPNEC that were resistant to platinum-based combination chemotherapy were recruited for a prospective phase II study of TMZ monotherapy. A 200 mg/m^2 dose of TMZ was given from day 1 to day 5, every 4 weeks. Response rate (RR) was evaluated as the primary end-point. The presence of O_6-methylguanine DNA methyltransferase (MGMT) in EPNEC patients was also evaluated as exploratory research. Thirteen patients were enrolled in this study. Primary lesions were pancreas (n = 3), stomach (n = 3), duodenum (n = 1), colon (n = 1), gallbladder (n = 1), liver (n = 1), uterus (n = 1), bladder (n = 1), and primary unknown (n = 1). Each case was defined as pathological poorly differentiated neuroendocrine carcinoma from surgically resected and/or biopsied specimens. The median Ki-67 labeling index was 60% (range, 22%-90%). The RR was 15.4%, progression-free survival was 1.8 months (95% confidence interval [CI], 1.0-2.7), overall survival (OS) was 7.8 months (95% CI, 6.0-9.5), and OS from first-line treatment was 19.2 months (95% CI, 15.1-23.3). No grade 3 or 4 hematological toxicity had occurred and there was one case of grade 3 nausea. One case presented MGMT deficiency and this case showed partial response. Temozolomide monotherapy is a feasible, modestly effective, and safe treatment for patients with unresectable EPNEC following platinum-based chemotherapy, especially those with MGMT deficiency.

KEYWORDS

extrapulmonary, neuroendocrine carcinoma, phase II study, poorly differentiated, temozolomide

Abbreviations: AE, adverse event; CI, confidence interval; CT, computed tomography; EPNEC, extrapulmonary neuroendocrine carcinoma; IHC, immunohistochemistry; LCNEC, large-cell neuroendocrine carcinoma; MGMT, O^6-methylguanine DNA methyltransferase; NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasm; NET, neuroendocrine tumor; OS, overall survival; PFS, progression-free survival; PS, performance status; RR, response rate; TMZ, temozolomide.

Trial registration: Registered 20 April 2013. Registry number: UMIN000010549.

1 | INTRODUCTION

Extrapulmonary poorly differentiated NEC originates in the gastrointestinal tract, and in gynecological and urological organs as either SCLC or LCNEC, which are both similar to their respective well-known pulmonary counterparts.[1] Extrapulmonary poorly differentiated NEC is a rare disease; its incidence accounts for 10%-20% of malignant NENs.[2,3] Extrapulmonary poorly differentiated NEC has aggressive histological features that contribute to its poor prognosis and lethality.[4] According to an analysis of 14 732 EPNEC cases from the Surveillance Epidemiology and End Results database, distant metastasis was diagnosed in 69% of patients with NEC, and the 5-year survival rate for patients with distant metastasis was only 5.7%.[5]

Systemic chemotherapy is the main treatment option for advanced EPNEC. Some clinical guidelines for treating EPNEC recommend platinum-based chemotherapy as the first-line treatment.[6,7] The response rate is approximately 30%-50%; however, the PFS is only 4 or 5 months.[8,9] The efficacy of platinum-based chemotherapy for EPNEC is limited, and second-line chemotherapy is necessary in one-half of all cases.[8,9]

To date, there has been no established second-line chemotherapy for EPNEC. Oxaliplatin-based chemotherapy, irinotecan-based chemotherapy and retreatment with platinum-based chemotherapy is retrospectively used as second-line chemotherapy; however, the response rate is 17%-40% and the PFS is only 1.9-4.8 months.[9,10,11] Amrubicin monotherapy is a promising second-line treatment for SCLC; therefore, it is the most frequently used second-line regimen for EPNEC, especially in Japan.[9,13,14] However, the response rate was 4.0%-38.5% and the PFS was 1.9-4.0 months.[9,12,13,14] Clinical classification of NENs has drastically changed during this decade; therefore, some cases of well-differentiated NETs with high Ki-67 index might have been classified as NEC in previously reported studies.

Temozolomide-based chemotherapy is one of the most frequently used second-line treatments for unresectable EPNEC.[8] The activity of TMZ in patients with unresectable EPNEC has been evaluated in several trials; however, most of them were retrospective studies or the study target was not limited to EPNEC.[15,16] Thus, the activity of TMZ for advanced EPNEC is still unclear. Moreover, there is no evidence of the efficacy of TMZ for Japanese patients with advanced EPNEC because TMZ is not yet approved in the Japanese health insurance system for use in advanced EPNEC. Prospective clinical study of TMZ monotherapy in Japanese patients with advanced EPNEC is a worthy challenge.

The aim of this study was to determine the efficacy and safety of TMZ monotherapy in patients with unresectable EPNEC resistant to platinum-based chemotherapy. Evaluation of the expression of MGMT, a chemosensitivity marker of TMZ, was also undertaken as an additional study.

2 | MATERIAL AND METHODS

2.1 | Study design

A phase II study using TMZ monotherapy as a second-line treatment in patients with unresectable EPNEC resistant to platinum-based chemotherapy was carried out from April 2013 to March 2017 (UMIN000010549) (IRB B130307033 and B160101021). Temozolomide was not approved for EPNEC by Japanese insurance support; therefore, we informed the efficacy, side-effects, and financial support organization for this clinical trial and obtained consent from all patients. Drug supply and funding support were provided by the Advanced Treatment Supportive Organization at Yokohama City University Hospital. This trial finished before March 2017; therefore, it did not meet the specifications of a special clinical trial.

The prevalence of MGMT deficiency in EPNEC was evaluated, and a correlation was found between MGMT deficiency and treatment response to TMZ by IHC as an exploratory research. This trial was supported by Yokohama City University Hospital.

2.2 | Patient selection

Patients with pathologically confirmed, metastatic, or recurrent poorly differentiated NEC (WHO 2010 Ki-67 labeling index greater than 20% for grade 3 tumors), who were previously treated with platinum-based first-line chemotherapy, were eligible for this study if they met the following inclusion criteria: ECOG PS of 0 or 1; age between 20 and 75 years; EPNEC with at least one measurable lesion based on RECIST; and adequate hematological, liver, and renal function (hemoglobin > 9.0 g/dL, white blood cell count <10 000/mm^3 and >3000/mm^3, neutrophil count >1500/mm^3, platelet count >100 000/mm^3, total bilirubin less than 1.5-fold the upper normal limit, serum transaminase less than three-fold the upper normal limit, and creatinine less than 1.5-fold the upper normal limit). The patients provided written informed consent. We excluded primary lung cancer (eg, SCLC and LCNEC), pathological diagnosis of mixed neuroendocrine neoplasm, adenocarcinoma with neuroendocrine features, and well-differentiated neuroendocrine tumor, so-called NET G3.

2.3 | Treatment

This was an open-label, single-center, non-randomized phase II study. All laboratory tests required to assess eligibility were completed within 7 days prior to the start of treatment. The treatment schedule involved the administration of TMZ (200 mg/m^2) on days 1-5 every 4 weeks. The RR was evaluated as the primary end-point, and the PFS, OS, and AEs were evaluated as secondary end-points. Computed tomography examinations were carried out every two

cycles. Adverse events were defined using the Common Terminology Criteria for Adverse Events version 4.0.

2.4 | Immunohistochemistry

Immunohistochemistry was undertaken on formalin-fixed, paraffin-embedded tissue sections. We reacted 4-μm-thick sections of representative blocks with mAbs against MGMT (MT 3.1; 1:25, GeneTex). Nuclear MGMT expression was scored as either intact or deficient in tumor cells.[17] Tumors were scored as intact when nuclear staining for MGMT was observed in any of the tumor cells. Tumors were scored as deficient when nuclear staining for MGMT was not observed in any tumor cells. Nonneoplastic cells served as an internal positive control in all tissue sections.

2.5 | Statistical design

SWOG's standard design (attained design) was used to determine the number of patients enrolled in the study. The null hypothesis stated that the overall RR would be less than 5% and the alternative hypothesis stated that the overall RR would be greater than 30%; the α error was determined to be 5% (one-tailed), and the β error was determined to be 20% (one-tailed). The alternative hypothesis was established based on data from previous reports. The sample size was set to 13 cases. The median survival time and corresponding 95% CIs for OS and PFS were estimated using the Kaplan-Meier method. Progression-free survival was defined as the time from day 1 of cycle 1 until the first event (progressive disease or death by any cause). If no such event occurred, data for that patient were censored on the day of the last imaging procedure. Overall survival was defined as the time from day 1 of cycle 1 until death by any cause. If death did not occur, data were censored on the last day of survival confirmation. All analyses were undertaken using SPSS version 21.0 (IBM).

3 | RESULTS

3.1 | Patients and characteristics

Between April 2013 and March 2017, 13 patients were enrolled in this study. Table 1 shows the patient characteristics at baseline. The median age of patients was 65 years (range, 40-75 years). Six patients were male and seven were female. Three patients had a PS of 1, and 10 cases had a PS of 0. All cases showed metastatic and unresectable EPNEC. Four patients underwent surgical resection of the tumor prior to treatment. The first-line chemotherapy regimens were irinotecan + cisplatin (n = 8), cisplatin + etoposide (n = 4), and carboplatin + etoposide (n = 1). Primary lesions were classified as pancreas (n = 3), stomach (n = 3), duodenum (n = 1), colon (n = 1), gallbladder (n = 1), liver (n = 1), uterus (n = 1), bladder (n = 1), and primary unknown (n = 1). The median Ki-67 labeling index was 60% (range, 22%-90%).

3.2 | Efficacy

A total of 28 cycles of TMZ were completed, and the median number of cycles per patient was two (range, 1-4). The RR was 15.4% and disease control rate was 23.1% (complete response = 0, partial response = 2, stable disease = 1, progressive disease = 10) (Table 2). Progression-free survival was 1.8 months (95% CI, 1.0-2.7) and OS was 7.8 months (95% CI,

TABLE 1 Characteristics of patients with extrapulmonary poorly differentiated neuroendocrine carcinoma treated with temozolomide

Case no.	Age (y)	Primary lesion	Metastasis lesions	Ki-67 index (%)	Initial treatment	Pretreatment times (mo)
1	60s	Stomach	Liver	80	IP	17.2
2	60s	Stomach	Liver	50	IP	6.7
3	60s	Stomach	Liver	70	IP	12.1
4	70s	Duodenum	LN, bone	70	Palliative surgery	7.1
5	60s	Rectum	Liver	70	EP	4.2
6	40s	Pancreas	Liver	22	GEM + erlotinib	9.8
7	40s	Pancreas	Liver	25	IP	82.8
8	70s	Pancreas	LN, peritoneum	34	IP	9.8
9	70s	Liver	Liver, lung	90	Surgical resection	10.6
10	40s	Gallbladder	Liver	30	CE	5.1
11	70s	Bladder	LN	69	IP	5.9
12	40s	Uterus	LN, bone	50	CDDP + radiation	19.4
13	60s	Primary unknown	Lung, bone	60	Palliative surgery	18.5

Abbreviations: CDDP, cisplatin; CE, etoposide plus carboplatin; EP, etoposide plus cisplatin; GEM, gemcitabine; IP, irinotecan plus cisplatin; LN, lymph node.

TABLE 2 Efficacy of temozolomide (TMZ) monotherapy in patients with extrapulmonary poorly differentiated neuroendocrine carcinoma

Case no.	Best response	Treatment cycle	PFS (mo)	OS (mo)	OS (1st line) (mo)	Post TMZ treatment	MGMT expression
1	PD	1	0.5	3.3	20.5	NT	NA
2	PD	2	1.8	6.9	13.6	S-1	Intact
3	PR	3	3.3	4.5	16.7	NT	Intact
4	PD	2	0.7	13.1	20.2	NT	Intact
5	PD	1	0.6	1.5	5.8	NT	Intact
6	PD	2	0.7	2.5	12.3	NT	Intact
7	PD	2	2.0	32.6	115.4	NT	NA
8	SD	4	3.8	8.4	18.2	Everolimus	Intact
9	PR	3	3.3	8.6	19.2	CapeOX + Bmab	Deficient
10	PD	2	0.8	7.1	12.0	NT	Intact
11	PD	2	1.6	33.7	39.5	AMR	Intact
12	PD	2	1.8	9.0	28.4	Topotecan	Intact
13	PD	2	1.8	8.9	27.4	AMR	Intact

Abbreviations: AMR, amrubicin; Bmab, bevacizumab; CapeOX, capecitabin plus oxaliplatin; MGMT, O^6-methylguanine DNA methyltransferase; NA, not available; NT, no treatment; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

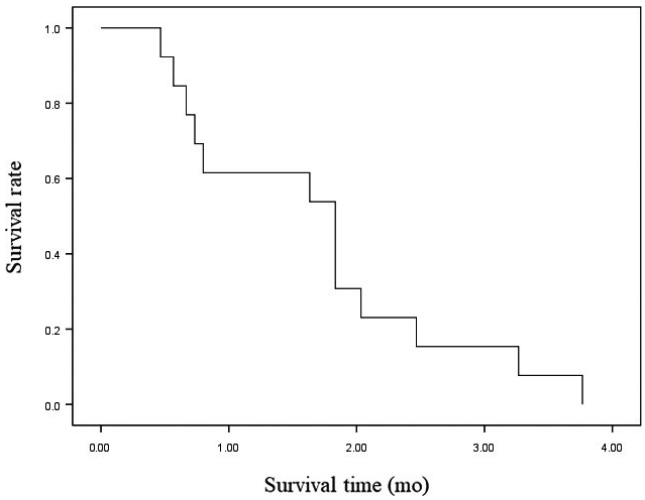

FIGURE 1 Kaplan-Meier curve for progression-free survival among patients with extrapulmonary neuroendocrine carcinoma treated with temozolomide monotherapy. The median progression-free survival was 1.8 months (95% confidence interval, 1.0-2.7 months). No patient data were censored

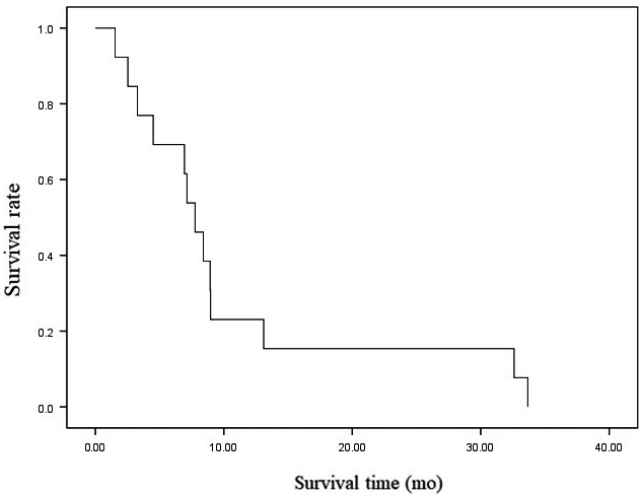

FIGURE 2 Kaplan-Meier curve for overall survival among patients with extrapulmonary neuroendocrine carcinoma treated with temozolomide monotherapy. The median overall survival was 7.8 months (95% confidence interval, 6.0-9.5 months). No patient data was censored

6.0-9.5) months. Overall survival from first-line treatment was 19.2 months (95% CI, 15.1-23.3) (Figures 1 and 2). In previously reported studies, high proliferation cases were highly responsive to platinum-based chemotherapy. Therefore, we separated patients into two groups according to Ki-67 labeling index: high proliferation group, Ki-67 50% or higher; and low proliferation group, Ki-67 less than 50%. Partial response was achieved in two cases (22.2%) in the high proliferation group. There was no response in the low proliferation group. However, PFS (high proliferation group, 1.8 months; 95% CI, 2.0-1.7 months vs low proliferation group, 0.8 months; 95% CI, 2.1-0 months; log rank $P = .488$), and OS was not significantly different in these two groups (high proliferation group 7.8 months; 95% CI, 10.2-5.3 months vs low proliferation group 7.1 months; 95% CI, 12.9-1.4 months; log rank $P = .739$).

3.3 | Toxicity

The most common AEs were hematological toxicities. There were no occurrences of grade 3 or 4 severe neutropenia, anemia, or thrombocytopenia. All hematological toxicities were nonsevere (grade 1 or 2); anemia occurred in 12 patients (92%), thrombocytopenia

occurred in five patients (39%), and leucopenia occurred in one patient (8%) (Table 3).

Of all severe nonhematological toxicities, only severe nausea was observed in one patient (8%) (Table 3). Grade 1 and 2 nonhematological toxicities that occurred included liver dysfunction in three patients (23%), renal dysfunction in three patients (23%), nausea in five patients (38%), vomiting in three patients (23%), diarrhea in one patient (8%), and general fatigue in four patients (31%).

TABLE 3 Hematological and nonhematological toxicities in patients with extrapulmonary poorly differentiated neuroendocrine carcinoma treated with temozolomide (N = 13)

	Grade 3, 4 n (%)	All grades n (%)
Anemia	0 (0)	12 (92)
Leucopenia	0 (0)	1 (8)
Neutropenia	0 (0)	0 (0)
Thrombocytopenia	0 (0)	5 (39)
Febrile neutropenia	0 (0)	0 (0)
Liver dysfunction	0 (0)	3 (23)
Renal dysfunction	0 (0)	3 (23)
Fever	0 (0)	1 (4)
Nausea	1 (8)	4 (31)
Vomiting	0 (0)	3 (23)
Diarrhea	0 (0)	1 (8)
General fatigue	0 (0)	4(31)

3.4 | Immunohistochemical analysis

Formalin-fixed, paraffin-embedded tissue sections were obtained from all cases. Sufficient tissues for immunohistochemical analysis could not be obtained from two cases. As a result, 11 tissue samples were analyzed in total (Table 2). Ten cases were found to be intact and one case was found to be deficient (Figure 3A,B). The deficient case had a primary liver NEC with multiple liver metastases and temporarily achieved a partial response (Figure 3C,D).

4 | DISCUSSION

The activity of TMZ in patients with unresectable NEN has been evaluated in several trials, which showed interesting activity in terms of RR values, ranging from 14% to 70%.[18-20] However, these clinical data describe TMZ-based chemotherapy for mainly well-to-moderately differentiated NETs (WHO 2019: grade1 and/or grade 2). Primary pancreatic NET shows a good response (43%–70%) to TMZ therapy with a long PFS rate (12-18 months).[19,20] Recently, in a prospective randomized phase II study for pancreatic NET (G1 or G2) with TMZ monotherapy vs capecitabine and TMZ combination

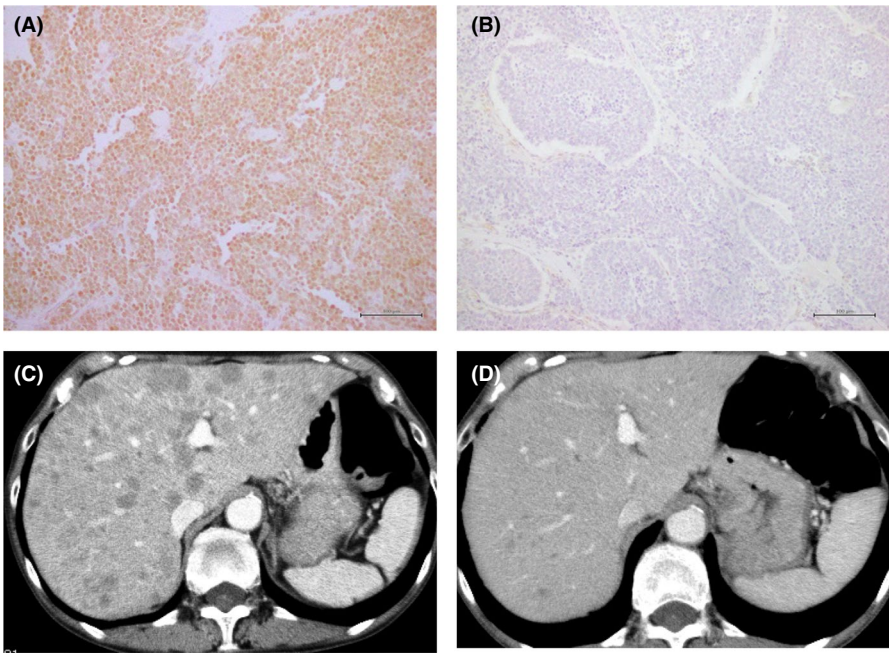

FIGURE 3 A, Serial section of a primary lesion of gastric neuroendocrine carcinoma with multiple liver metastases (case 3). Immunohistochemical findings revealed tumor cells were diffuse and moderately stained by O_6-methylguanine DNA methyltransferase (MGMT) protein. This case was defined as intact. B, Serial section of a liver lesion of hepatic neuroendocrine carcinoma (case 9). Immunohistochemical findings revealed tumor cells were not stained by MGMT protein diffusely. This case was defined as deficient. C, Computed tomography findings revealed multiple liver metastases before treatment with temozolomide (case 9). D, Computed tomography findings revealed multiple liver metastases after two treatment cycles with temozolomide. Multiple liver tumors showed remarkable shrinkage (case 9)

chemotherapy, the response rate was 33.3% vs 27.8%, respectively, and the PFS was 22.7 months vs 14.4 months. Temozolomide and capecitabine combination chemotherapy shows a significantly better PFS than TMZ monotherapy.[21] The ENETS consensus guidelines describing TMZ-based chemotherapy should be considered for well-differentiated NEN, including NET G3.[6]

However, TMZ-based chemotherapy for EPNEC is not sufficiently effective as an urgent treatment. In a previous study, TMZ monotherapy or TMZ in combination with capecitabine and bevacizumab for EPNEC showed a response rate of 0%–57% and a PFS of 2.4-8.4 months.[15,16,22]

In general, second- or third-line chemotherapy treatment for EPNEC has a response rate of 0%-18.8% and a PFS of 2.1-3.8 months.[9,14,16,23] In this study, we estimated the response rate to be 15.4% and the PFS as 1.8 months. Here, TMZ monotherapy was undertaken in six cases as a second-line treatment, but in the other seven cases, it was undertaken as the third- or salvage-line treatment. Patients in this study undergoing TMZ monotherapy showed more advanced disease than patients from the other TMZ studies reported so far. According to recently published real-world data, the median PFS for poorly differentiated NEC was 2.8 months in the TMZ monotherapy group and 2.9 months in the capecitabine combination group ($P = .982$). Median OS for poorly differentiated NEC was 6.2 months in the TMZ monotherapy group and 12.7 months in the capecitabine combination group ($P = .613$). Capecitabine addition did not have a significant impact on EPNEC, compared with NET. The addition of other agents to TMZ might be necessary for EPNEC. Temozolomide monotherapy is mildly toxic, and incidents of severe toxic events have been rare in previously reported studies. In this study, there were no grade 3 or 4 hematological and few nonhematological toxicities. Patients received chemotherapy for a long duration (approximately 6 months; detailed data not shown), and their general condition was no better than that reported in previous studies. No severe toxicity was observed in these advanced patients. Recently, many studies for unresectable NEN were undertaken using TMZ and capecitabine combination therapy that showed the rate of severe adverse events was very low (grade 3 or 4 hematological toxicities, 3.4%; nonhematological toxicities, less than 1%) and confirmed the mild toxicity of this therapy.[25]

O^6-methylguanine DNA methyltransferase deficiency was confirmed by IHC analysis, and a case of deficiency in response to TMZ monotherapy was found in this study. Expression of MGMT in tumor cells was assessed as a predictive biomarker for alkylating agents. One mechanism of resistance to alkylating agents is an increase in the expression of the DNA repair enzyme MGMT. If the expression of MGMT is decreased by methylation of the *MGMT* promoter region, DNA repair is decreased. A decrease in MGMT expression, which frequently occurs during carcinogenesis, could increase the sensitivity of tumor cells to alkylating agents that induce DNA damage, thus increasing the response to alkylating agents. Many previous studies have described the relationship between MGMT expression and the response of NENs.[17,26] In our study, the analysis of the MGMT-deficient case showed shrinkage of multiple liver tumors after two treatment cycles. However, the tumors rapidly regrew after two cycles. According to another recent study, metronomic TMZ monotherapy (one-week-on/one-week-off treatment) for pancreatic NEC with MGMT deficiency is a good option for second-line chemotherapy.[27] We consider TMZ monotherapy as a potential treatment option for EPNEC with MGMT deficiency as a second- or third-line treatment.

This study has some limitations. First, the sample size was small, and the primary lesions were heterogeneous. Most prior studies were designed for unresectable pancreatic NEN or GEP-NEN. Extrapulmonary NEC shows a high degree of heterogeneity, so the efficacy of TMZ could differ according to primary lesions. Second, we evaluated MGMT status using IHC. In a clinical setting, MGMT status can be evaluated by assessing MGMT protein expression using IHC or by determining MGMT promoter methylation using a methylation assay. Some previous reports suggest that concordance is relatively low between IHC and methylation-specific PCR assessments of MGMT status.[28]

Our study revealed that salvage-line TMZ monotherapy was a safe and marginally effective treatment for patients with unresectable EPNEC after failure of first-line platinum-based chemotherapy. We consider TMZ monotherapy as a potential treatment option for EPNEC with MGMT deficiency as a second- or third-line treatment. A more effective second-line treatment option is necessary for EPNEC, but chemotherapy with TMZ combined with another agent could serve as a feasible and effective treatment option for EPNEC treatment, especially in the presence of MGMT deficiency.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICAL APPROVAL

This study was conducted in accordance with the principles expressed in the Declaration of Helsinki. The main protocol of this study was approved by the institutional review board of Yokohama City University (Trial No. B130307033 and B160101021) at March 8th 2013 and February 5th 2016. Protocol amendments have been made in the study period. The latest version (version 3.0) of study protocol has been reviewed and approved by the institutional review board in Yokohama City University. Written informed consent will be obtained from all the participants.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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