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Efficacy and safety of ramucirumab-containing chemotherapy in patients with pretreated metastatic gastric neuroendocrine carcinoma

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ABSTRACT

Background Ramucirumab (RAM), a monoclonal antibody for vascular endothelial growth factor 2 (VEGFR2), has been effective for advanced gastric adenocarcinoma (AC). However, little is known about the efficacy of RAM-containing chemotherapy (RAM-CTx) in gastric neuroendocrine carcinoma (G-NEC).

Methods We retrospectively analysed and compared the clinical outcomes of patients (pts) with G-NEC receiving RAM-CTx, G-NEC receiving CTx without RAM and AC receiving RAM-CTx in our hospital. G-NEC was defined by neuroendocrine carcinoma features, regardless of the proportion, based on histology and neuroendocrine markers (synaptophysin, chromogranin A or CD56). VEGFR2 expression in tumour vessels was evaluated in archival primary G-NEC tissues by immunohistochemistry using the same anti-VEGFR2 primary antibody and scoring scheme (vascular VEGFR2 H-score) as in the REGARD trial.

Results Seventeen G-NEC receiving RAM-CTx, 13 G-NEC receiving CTx without RAM and 173 AC pts receiving RAM-CTx were analysed. The overall response rate (59% vs 8 % vs 28%), progression-free survival (median 7.7 vs 1.8 vs 3.3 months) and overall survival (median 16.1 vs 8.6 vs 9.6 months) were significantly better in pts with G-NEC receiving RAM-CTx than G-NEC receiving CTx without RAM or AC receiving RAM-CTx. No severe or unexpected adverse events occurred. The median vascular VEGFR2 H-score, based on available G-NEC tissues from 12 pts receiving RAM-CTx, was 220 (range 150–260), which was markedly higher than that reported on AC tissues from the REGARD trial as historical control (median 35, range 0–240).

Conclusions RAM-CTx showed a promising activity without severe or unexpected safety profile in pts with G-NEC. This may in part be explained by higher vascular VEGFR2 expression in G-NEC tissues.

Key questions

What is already known about this subject?
- Gastric neuroendocrine carcinoma (G-NEC) is a poorly differentiated carcinoma with high cellular proliferation activity among neuroendocrine tumours.
- G-NEC is known to be associated with poor prognosis.
- Standard chemotherapies for patients with metastatic G-NEC are not established.

What does this study add?
- Ramucirumab-containing chemotherapy showed a promising activity in patients with G-NEC.
- Vascular endothelial growth factor receptor 2 expression in G-NEC tissues was extremely high.

How might this impact on clinical practice?
- Our data might lead to new treatment strategy for G-NEC.

INTRODUCTION

Neuroendocrine carcinoma (NEC) is a poorly differentiated carcinoma with high cellular proliferation activity among neuroendocrine tumours.[1] Gastric NEC (G-NEC), accounting for 6.0%–7.8% of gastric tumours,[2 3] is associated with poor prognosis due to high frequency of haematological and lymphatic metastases.[4 5] Although standard chemotherapies for patients (pts) with metastatic G-NEC are not established, platinum-containing chemotherapy is usually selected as first-line treatment based on clinicopathological similarities between extrapulmonary NEC and small cell lung cancer (SCLC).[6] The median overall survival (OS) of pts with G-NEC treated with chemotherapy is approximately 1 year.[6 7] Efficacies of salvage treatments for G-NEC are limited.[6–9] The overall response rate (ORR), median progression-free survival (PFS) and median OS in pts with extrapulmonary NEC who received amrubicin were 4%–38.5%, 1.9–3.5 months and 7.1–8.3 months, respectively.[7–9]

Additionally, the ORR and median PFS in pts with G-NEC who received taxanes or temozolomide were reportedly 18% and 3 months, respectively.[10]

Neuroendocrine neoplasms, including NEC, are highly vascular tumours, and inhibition of angiogenesis has been a promising strategy.[11] Sunitinib, which inhibits the tyrosine kinase of vascular endothelial growth factor (VEGF) receptors, improved OS compared with placebo among pts with advanced pancreatic neuroendocrine tumours.[12] The addition of bevacizumab, a monoclonal antibody for VEGF-A, to cisplatin and etoposide in the first-line treatment of SCLC led to statistically significant improvement in PFS.[13] Ramucirumab (RAM) is a fully human IgG_1 monoclonal antibody to the extracellular binding domain of VEGF receptor 2 (VEGFR2), inhibiting VEGF ligand binding and receptor signalling and limiting VEGF-induced angiogenesis and migration of endothelial cells. Recently, the REGARD and RAINBOW trials proved that RAM is effective for advanced gastric adenocarcinoma (AC).[14 15] Biomarker analysis in the REGARD trial showed that the efficacy of RAM appeared more pronounced in pts with high VEGFR2 expression.[16] This suggested activity of RAM for pts with G-NEC. However, little is known about the efficacy of RAM-containing chemotherapy (RAM-CTx) and VEGFR2 expression in G-NEC.

In this study, we investigated the efficacy and safety of RAM-CTx and VEGFR2 expression in pts with G-NEC.

PATIENTS AND METHODS

Patients

This retrospective study evaluated the efficacy and safety of RAM-CTx in pts with pretreated G-NEC. We reviewed the medical records of consecutive pts with G-NEC who were treated with RAM-CTx in a single institution between June 2015 and January 2017. The clinical outcomes were compared with those of pts with G-NEC receiving CTx without RAM between June 2012 and January 2017 or pts with AC receiving RAM-CTx between June 2015 and January 2017.

The eligibility criteria were presence of histologically proven, metastatic G-NEC or AC; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; adequate bone marrow, hepatic and renal function; previous treatment with one or more regimens; and at least one treatment with RAM-CTx. Pathological diagnoses of G-NEC for all surgical/biopsy specimens were made based on morphological and immunohistochemical (IHC) findings according to the WHO classification[1] at the Department of Pathology in National Cancer Center Hospital East. NEC markers including chromogranin A, synaptophysin, CD56 and Ki-67 were employed for IHC analysis, and any tumour showing convincing positivity for at least one of the NEC markers, regardless of the proportion, was considered G-NEC. All the specimens were reviewed by TK and SM for this study.

IHC assays for VEGFR2 and CD34

Using a well-characterised, commercially available antibody and specific, selective and sensitive IHC assay,[17] VEGFR2 protein expression was visualised in 5 µm sections prepared from G-NEC primary formalin-fixed paraffin-embedded (FFPE) tumour specimens of pts receiving RAM-CTx and quantified as H-score (range, 0–300) as in the REGARD trial,[16] based on the intensity and proportion of unequivocally stained tumour vessels. Similarly, CD34 was used to localise tumour stromal vessels by IHC in 5 µm sections of FFPE G-NEC specimens using well-established staining protocols in a CLIA lab and a CD34 scoring scheme (range, 0–3), based on the proportion of stained tumour vessels.

Outcomes and statistical analysis

We assessed ORR, disease control rate (DCR), PFS and OS. Tumour response was assessed in pts with measurable lesions according to the guidelines of the Response Evaluation Criteria in Solid Tumours V.1.1. ORR was defined as the proportion of pts with the best overall response of complete response (CR) or partial response (PR). DCR was defined as the proportion of pts with the best overall response of CR, PR or stable disease. PFS was defined from the date of initiation of chemotherapy to the date of disease progression or death from any cause. OS was estimated from the date of initiation of chemotherapy to the date of death or last follow-up visit. Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, V.4.0.

χ^2 test or Fisher's exact test was used to compare categorical variables, baseline characteristics and response rates. PFS and OS rates were estimated by the Kaplan-Meier method and compared between G-NEC and AC by univariate and multivariate analyses using Cox proportional hazards models and presented as HRs with 95% CIs. In multivariate analyses, forward and backward stepwise methods were used for model building using threshold p values of 0.10 for inclusion and 0.20 for exclusion. Confounding variables considered in the multivariate analyses were age (<median vs median or older), gender (male vs female), ECOG PS (0–1 vs 2), prior gastrectomy (no vs yes), number of previous line (1 versus ≥2), liver metastases (no vs yes), peritoneum metastases (no vs yes), number of metastases (1–2 versus ≥3) and regimen of RAM-CTx (RAM plus paclitaxel vs RAM plus irinotecan or RAM monotherapy). Statistical analyses were performed using SPSS Statistics V.21 software. All tests were two-sided; $p<0.05$ was considered to indicate statistical significance.

Vascular VEGFR2 expression and Ki-67 index were categorised as low or high using the median score value as the cut-off. CD34 expression was also categorised as low or high using the score (0–2+ vs 3+). PFS or OS among the groups according to VEGFR2, Ki-67 and CD34 status were compared using Cox proportional hazards models and presented as HRs with 95% CIs. Vascular VEGFR2 H-score was divided into four score groups (0: H-score

0; 1: H-score 1–100; 2: H-score 101–200; and 3: H-score 201–300). For each specimen, vascular VEGFR2 score was added to CD34 score to calculate the combined VEGFR2-CD34 index. The relationship between PFS and combined VEGFR2-CD34 index was assessed using bean plots.

RESULTS

Patient characteristics

Seventeen G-NEC receiving RAM-CTx (G-NEC with RAM), 13 G-NEC receiving CTx without RAM (G-NEC without RAM) and 173 AC pts receiving RAM-CTx (AC with RAM) who met the eligibility criteria were analysed. In G-NEC with RAM, RAM-CTx included RAM plus paclitaxel (n=13, 76%), RAM plus irinotecan (n=2, 12%) and RAM monotherapy (n=2, 12%), which did not significantly differ in AC with RAM. In G-NEC without RAM, CTx included amrubicin (n=6, 46%), irinotecan (n=4, 31%) and paclitaxel (n=3, 23%). Fifteen pts in G-NEC with RAM (88%), 10 pts in G-NEC without RAM (100%) and 148 pts in AC with RAM (86%) were previously treated with platinum-based chemotherapy as first-line. Ki-67 was assessed in 15 pts in G-NEC with RAM and 11 pts in G-NEC without RAM; its median index was 75% (range, 50%–95%) and 80% (range, 20%–95%), respectively. Pts in G-NEC with RAM were associated with significantly higher frequencies of male (100% vs 54% vs 64%, p<0.01) and elderly (p=0.03). Pts in G-NEC with RAM

Table 1 Patient characteristics

	G-NEC			
	RAM (n=17) (%)	Without RAM (n=13) (%)	AC (n=173) (%)	P values
Age				
Median (range)	74 (53–85)	64 (57–73)	67 (24–84)	0.03
Gender				
Male	17 (100)	7 (54)	110 (64)	<0.01
ECOG PS				
0	14 (82)	11 (85)	109 (63)	0.1
1	2 (12)	2 (15)	55 (32)	
2	1 (6)	0 (0)	9 (5)	
Ki-67				
Median (n, range)	75%(15, 50–95%)	80% (11, 20%–95%)		
Missing (n)	(2)	(2)		
Previous gastrectomy				
Yes	5 (29)	4 (31)	39 (23)	0.67
Number of previous treatment				
1	13 (76)	12 (92)	117 (68)	0.13
≥2	4 (24)	1 (8)	56 (32)	0.13
Target lesion				
Yes	17 (100)	13 (100)	135 (78)	0.02
Site of metastases				
Liver	11 (65)	9 (70)	58 (34)	<0.01
Lymph nodes	11 (65)	13 (100)	99 (57)	<0.01
Lung	1 (6)	0 (0)	15 (9)	0.51
Peritoneum	0 (0)	0 (0)	86 (50)	<0.01
Number of metastases				
1–2	17 (100)	11 (85)	150 (87)	0.33
≥3	0 (0)	2 (15)	23 (13)	
Chemotherapy	RAM+paclitaxel 13 (76) RAM+irinotecan 2 (12) Rmonotherapy 2 (12)	Amrubicin 6 (46) Irinotecan 4 (31) Paclitaxel 3 (23)	RAM+ paclitaxel 126 (73) RAM+ irinotecan 25 (14) RAMmonotherapy 22 (13)	0.76

AC, adenocarcinoma; ECOG PS, Eastern Cooperative Oncology Group performance status; G-NEC , gastric neuroendocrine carcinoma; RAM, ramucirumab.

Table 2 Tumour response in measurable lesions

	G-NEC			
	RAM (n=17)	Without RAM (n=13)	AC (n=135)	P values
Complete response, n (%)	0 (0)	0 (0)	0 (0)	
Partial response, n (%)	10 (59)	1 (8)	38 (28)	
Stable disease, n (%)	5 (29)	5 (38)	66 (49)	
Progressive disease, n (%)	1 (6)	7 (54)	29 (21)	
Objective response rate (%)	59	8	28	<0.01
Disease control rate (%)	88	46	77	0.04
Not evaluable/not assessed, n (%)	1 (6)	0 (0)	2 (1)	

AC, adenocarcinoma; G-NEC, gastric neuroendocrine carcinoma; RAM, ramucirumab.

and G-NEC without RAM were associated with significantly higher frequencies of liver metastasis (65% vs 70% vs 34%, p<0.01), while those in AC with RAM were associated with significantly high incidence of peritoneal metastasis (0% vs 0% vs 50%, p<0.01). There was no other significant difference (table 1).

Efficacy and safety of treatment

In 17 pts in G-NEC with RAM, 10 achieved PR with an ORR of 59%. The ORR (59% vs 8% vs 28%, p<0.01) and DCR (88% vs 46% vs 77%, p=0.04) were significantly higher in G-NEC with RAM than in G-NEC without RAM or AC with RAM (table 2 and online supplementary figure s1). Notably, five pts with G-NEC (29%) received RAM-CTx for more than 1 year (online supplementary figure s1).

PFS was significantly longer in G-NEC with RAM than in G-NEC without RAM or AC with RAM (median 7.7 months, 1.8 months and 3.3 months; figure 1). The HR for PFS in G-NEC with RAM compared with that in G-NEC without RAM or AC with RAM was 0.18 (95% CI 0.07 to 0.49; p<0.01) and 0.39 (95% CI 0.22 to 0.67; p<0.01), respectively. After adjustment by confounding factors, the HR for PFS in G-NEC with RAM compared with that in AC with RAM was 0.49 (95% CI 0.26 to 0.91; p=0.02). All pts in G-NEC with RAM have received RAM-CTx after

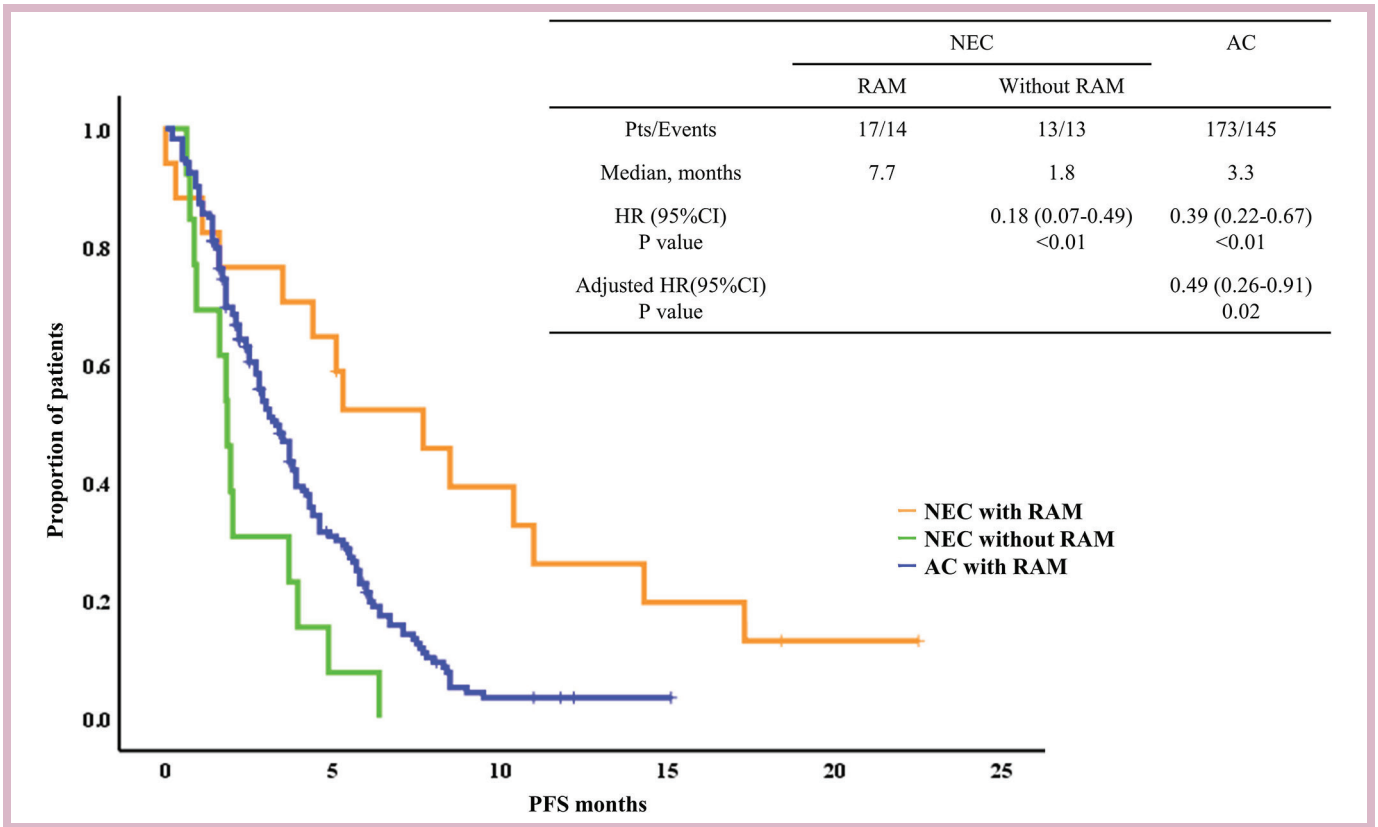

Figure 1 Kaplan-Meier curves of progression-free survival (PFS). PFS was significantly longer in G-NEC with RAM than in G-NEC without RAM and AC with RAM. (*Adjusted by multivariate analysis using stepwise method.) AC, adenocarcinoma; G-NEC, gastric neuroendocrine carcinoma; NEC, neuroendocrine carcinoma; pts, patients; RAM, ramucirumab.

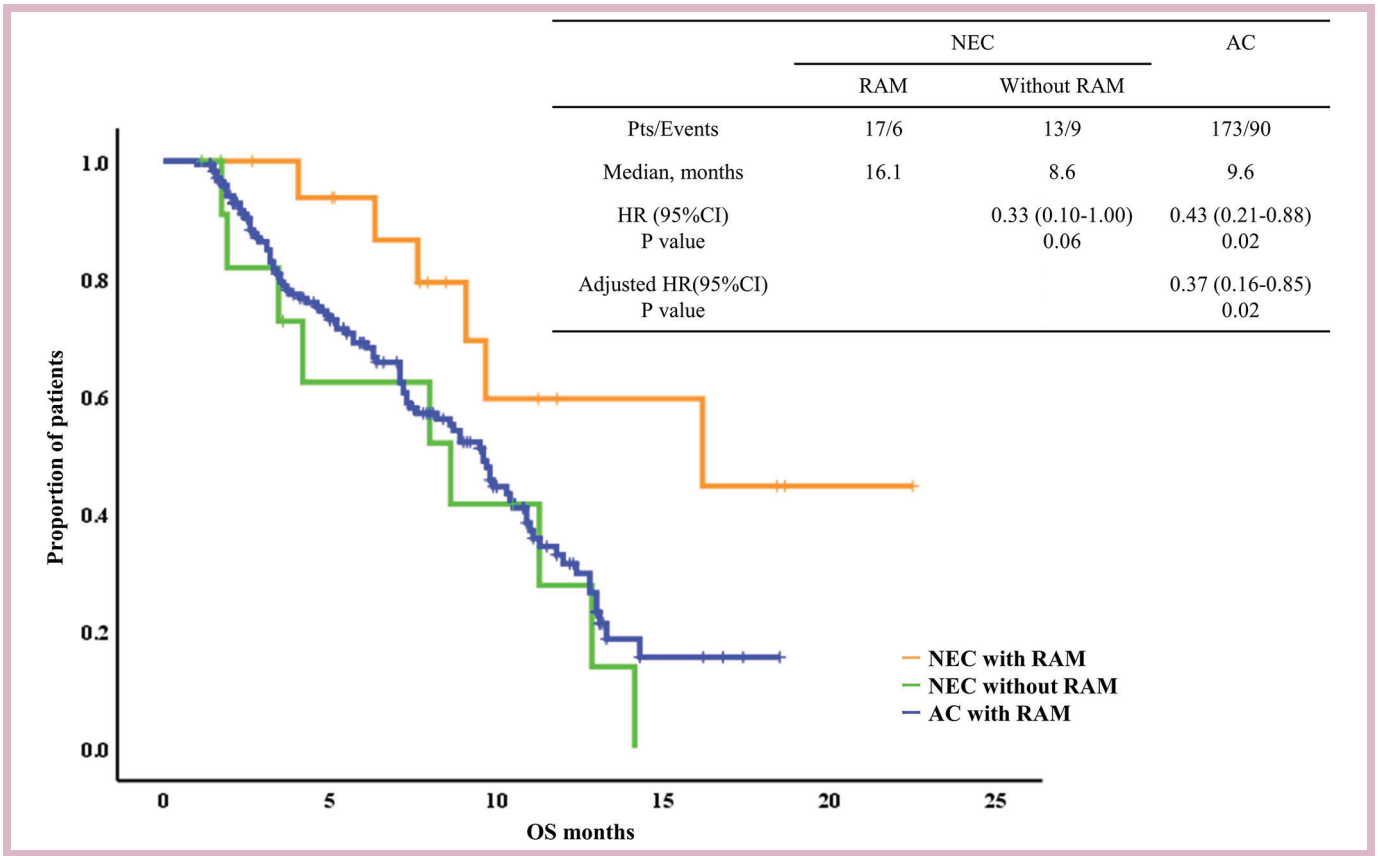

Figure 2 Kaplan-Meier curves of overall survival (OS). OS was also significantly longer in G-NEC with RAM than AC with RAM. (*Adjusted by multivariate analysis using stepwise method.) AC, adenocarcinoma; G-NEC, gastric neuroendocrine carcinoma; NEC, neuroendocrine carcinoma; pts, patients; RAM, ramucirumab.

being refractory to prior CTx. Fifteen pts received platinum-containing CTx as first-line (IRI+CDDP, n=9; SOX, n=6). The objective response rate and DCR for platinum-containing CTx were 67% and 93%, respectively. Among these pts, the PFS of RAM-CTx was longer than that of platinum-based chemotherapy in eight pts (53%) (online supplementary figure s3). On the other hand, among the 148 pts in AC with RAM receiving first-line platinum combinations, the PFS of RAM-CTx was longer than that of first-line only in 39 pts (27%) (online supplementary figure s3). The OS was significantly longer in G-NEC with RAM than in G-NEC without RAM or AC with RAM (median 16.1 months, 8.6 months and 9.6 months, respectively; figure 2), with median follow-up period of 9.9 months (range, 2.7–27.8 months) in pts with G-NEC with RAM, 4.2 months (range, 1.2–14.1 months) in pts with G-NEC without RAM and 8.6 months (range, 1.0–25.5 months) in those with AC. The HR for OS in G-NEC with RAM compared with that in G-NEC without RAM or AC with RAM was 0.33 (95% CI 0.10 to 1.0; p=0.06) and 0.43 (95% CI 0.21 to 0.88; p=0.02), respectively. After adjustment for confounding factors, the HR for OS in G-NEC with RAM compared with that in AC with RAM was 0.37 (95% CI 0.16 to 0.85; p=0.02).

Common grade 3 or worse adverse events in pts with G-NEC with RAM were neutropaenia (59%), thrombocytopaenia (6%), congestive heart failure (6%) and gastrointestinal perforation (6%), which were not significantly different in pts with AC (table 3). No severe or unexpected adverse events occurred.

Biomarker analyses

Vascular VEGFR2 and CD34 protein expression was assessed in available archival tumour tissue from 12 pts with G-NEC with RAM (71%) (online supplementary figure s4). The median vascular VEGFR2 H-score in G-NEC was 220 (range, 150–260), which was markedly higher than that reported on AC tissue in the REGARD trial (median, 35; range, 0–240).[16] Pts with G-NEC with high vascular VEGFR2 H-score (>median, n=6) had better OS than those with lower score (n=6, HR 0.28 (95% CI 0.31 to 2.5)), although there was no statistical significance due to the small patient numbers (online supplementary figure s5). No clear trend of difference of PFS was noted by vascular VEGFR2 H-score, CD34 score and Ki-67 index (online supplementary figure s6: A-C). Additionally, in scatter plots, no clear correlation was found between PFS and combined VEGFR2-CD34 index (online supplementary figure s6: D).

DISCUSSION

We retrospectively evaluated the efficacy and safety of RAM-CTx for pts with pretreated metastatic G-NEC. To

Table 3 RAM-containing chemotherapy-related adverse events occurring in at least 20% of patients and grade 3 or more adverse events occurring in at least 5% of patients

	G-NEC (n=17)		AC (n=173)	
	All (%)	Grades 3–4 (%)	All (%)	Grades 3–4 (%)
Neutropaenia	15 (88)	10 (59)	65 (43)	59 (34)
Anaemia	6 (35)	0 (0)	60 (35)	10 (6)
Thrombocytopaenia	4 (24)	1 (6)	13 (8)	4 (2)
Febrile neutropaenia	1 (6)	1 (6)	5 (3)	5 (3)
Peripheral oedema	8 (47)	0 (0)	37 (21)	0 (0)
Neuropathy	6 (35)	0 (0)	39 (23)	0 (0)
Liver injury or failure	8 (47)	0 (0)	43 (25)	0 (0)
Congestive heart failure	1 (6)	1 (6)	0 (0)	0 (0)
Gastrointestinal perforation	1 (6)	1 (6)	2 (1)	2 (1)

AC, adenocarcinoma; G-NEC, gastric neuroendocrine carcinoma.

our knowledge, this is the first report to provide information on the efficacy and safety of RAM-CTx for pts with pretreated G-NEC in comparison with G-NEC receiving CTx without RAM and AC receiving RAM-CTx.

In this study, RAM-CTx showed promising activity with ORR of 59% and median PFS of 7.7 months in pts with G-NEC without a severe or unexpected safety profile. Efficacy results of RAM-CTx seemed to be more favourable than those of salvage treatments using taxanes, amrubicin or irinotecan for G-NEC in our hospital and other reports,[7–10] although a cross-trial comparison should be cautiously interpreted based on different patient characteristics and small sample sizes. Furthermore, the PFS of RAM-CTx was longer than that of first-line in eight pts (53%), thereby supporting the efficacy of RAM-CTx for pts with G-NEC.

Retrospective exploratory biomarker analysis in the REGARD trial reported a more pronounced benefit of RAM in pts with high VEGFR2 expression (HR in OS 0.69, and PFS HR 0.35 in VEGFR high; and HR in OS 0.73, PFS HR 0.73 in VEGFR2 low), although statistically significant interactions were not confirmed.[16] The median vascular VEGFR2 H-score of G-NEC tissue in our study (median, 220; range, 150–260) was markedly higher than that reported in AC tissue in the REGARD trial (median, 35; range, 0–240),[16] which may be a reason for the high activity of RAM-CTx for pts with G-NEC. Even though the biological behaviour of VEGFR2 expression in NEC has not been completely understood, VEGFR2-specific intracellular signalling cascades are considered to be leading to proliferation, migration and survival.[18] In the REGARD trial, it has also been reported that high vascular VEGFR2 levels may be associated with earlier progression in the placebo arm (PFS; HR 1.65). In our study, higher vascular VEGFR2 expression in pts with G-NEC who were treated with RAM appeared to be associated with longer OS (HR 0.28, 95% CI 0.31 to 2.5), although it was not statistically significant. It suggests that RAM-CTx may be more effective in pts with G-NEC with higher vascular VEGFR2 expression, which warrants further evaluations in a larger cohort. We also evaluated CD34 as a marker of vascular endothelial progenitor cells to detect VEGFR2 expression specific for vessels. High Ki-67 index is known to be associated with poor prognosis in NEC.[19] In our pts cohort, PFS was not significantly different between high and low Ki-67 index, suggesting that RAM-CTx is effective even in pts with G-NEC who have a high Ki-67 index.

Our retrospective analysis has several limitations. This was a retrospective study in a single institution with a small sample size. Exploratory biomarker analysis was conducted in a limited number of samples. Ki-67 index was not obtained for 2 of 17 pts with G-NEC with RAM and 2 of 13 pts with G-NEC without RAM, although the diagnosis of G-NEC was confirmed by other measures. The diagnosis of G-NEC was performed using biopsied specimens in 15 of 17 pts; therefore, the proportion of NEC component could not be identified in most samples.

In conclusion, RAM-CTx was suggested to be active and well tolerated in pts with G-NEC. This may be explained in part by higher vascular VEGFR2 expression in G-NEC tissues. These results warrant further evaluations in a larger cohort. Also, if confirmed, it may warrant further evaluation in a randomised study to compare currently available other therapies for NEC, such as platinum-containing chemotherapy or amrubicin.

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Contributors SM, AK, HM and KS designed the study, collected the data, performed the data analysis and wrote the manuscript. YK, HB, TK, TD, AO and TY were involved in data interpretation and critically reviewing the manuscript. TK, EMN, SC and AN were involved in testing the tumour tissue as well as critically reviewing the manuscript. All authors read and approved the final manuscript.

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Patient consent Obtained.

Ethics approval This study was performed under an institutional review board waiver in accordance with the Japanese ethical guidelines for epidemiological research. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and informed consent.

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