Second-line treatment in patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma: a systematic review and meta-analysis

Mairéad G. McNamara, Melissa Frizziero, Timothy Jacobs, Angela Lamarca, Richard A. Hubner, Juan W. Valle and Eitan Amir

Abstract

Background: There is no standard second-line treatment for patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma (EP-PD-NEC). This study explored data evaluating second-line treatment in these patients.

Methods: A search of MEDLINE and EMBASE identified studies reporting survival and/or response data for patients with EP-PD-NEC receiving second-line therapy. Association between various factors (age, gender, ECOG performance status, primary tumour location, morphology, Ki-67, treatment and grade 3/4 haematological toxicity) and response rate (RR), progression-free (PFS) and overall survival (OS) were assessed with a mixed effects meta-regression weighted by individual study sample size. Due to a small sample size, associations were reported quantitatively, based on magnitude of beta coefficient rather than statistical significance.

Results: Of 83 identified studies, 19 were eligible, including 4 prospective and 15 retrospective studies. Analysis comprised 582 patients, with a median number of 19 patients in each study (range 5–100). Median age was 59 years (range 53–66). Median RR was 18% (range 0–50; 0% for single-agent everolimus, temozolomide, topotecan; 50% with amrubicin), median PFS was 2.5 months (range 1.15–6.0) and median OS was 7.64 months (range 3.2–22.0). Studies with a higher proportion of patients with a Ki-67 >55% had lower RR ($\beta = -0.73$) and shorter OS ($\beta = -0.82$).

Conclusion: Second-line therapy for patients with advanced EP-PD-NEC has limited efficacy and the variety of regimens used is diverse. Ki-67 >55% is associated with worse outcomes. Prospective randomised studies are warranted to enable exploration of new treatment strategies.

Keywords: advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma, meta-analysis, second-line treatment, survival, systematic review

Introduction

Extra-pulmonary (EP) neuroendocrine carcinoma (NEC) is an aggressive entity with poorly differentiated (PD) morphology; Ki-67 >20%, extensive necrosis, and nuclear atypia.\(^1\)\(^2\) Gastroenteropancreatic (GEP) NECs are rare and account for 5–10% of GEP neuroendocrine neoplasms.\(^3\) Primary EP-PD-NECs can arise in different organs, including the aerodigestive and genitourinary tract. However, in 7–30% of patients, the primary site is unknown.\(^4\)

Surgical resection is the only potentially curative approach, but patients present at an advanced stage in up to 85% of cases.\(^5\) First-line treatment for patients with advanced EP-PD-NEC, who
have a perceived similarity to small cell carcinoma of the lung, has remained unchanged since the early 1990s, when data showed that the etoposide/platinum combination produced anti-tumour activity and high tumour response rates (RRs). The median overall survival (OS) of patients with EP-PD-NEC treated with first-line platinum-based chemotherapy in the advanced setting is 11–16.4 months. In one of the largest retrospective series including patients with advanced gastrointestinal NEC, Sorbye et al. reported that the RR to first-line chemotherapy in 252 patients with advanced gastrointestinal NECs (the NORDIC NEC study) was 31%, and patients with a Ki-67 < 55% had a lower RR, but improved OS, indicating heterogeneity in survival outcomes within this disease group.

However, disease progression inevitably occurs, and there is no established second-line treatment for patients with advanced EP-PD-NEC, and hence effective therapies are urgently needed. In the NORDIC NEC study, the RR reported after second-line chemotherapy for 84 assessable patients was 18% (see Table 1 for details of additional studies reporting RR to second-line systemic therapy in this disease group). In the NORDIC NEC study, patients who had immediate progression on first-line chemotherapy had a median OS of 6 months. The European Neuroendocrine Tumour Society (ENETS) consensus guidelines concluded that second-line regimens for advanced high grade GEP NECs have not been rigorously evaluated, but that options such as temozolomide-, irinotecan- or oxaliplatin-based schedules could be considered.

Interestingly, a recent retrospective review from two ENETS Centres of Excellence reported that 113 patients received first-line systemic therapy for advanced EP-PD-NEC over approximately two decades, and only 31% went on to receive second-line treatment, with at least six differing regimens utilised, highlighting the uncertainty of choice regarding the most efficacious second-line regimen and the rarity of this population, presenting even to large tertiary referral centres.

The aim of this current study was to explore published data evaluating second-line systemic treatment in patients with advanced EP-PD-NEC, to inform potential future trial design.

**Methods**

**Data sources and search**

This analysis was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic review of electronic databases [MEDLINE (host: OVID) and EMBASE (host: OVID)] from 1996 to 31 October 2018 was supplemented by a manual search of the American Society of Clinical Oncology abstracts 2016–2018 and European Society of Medical Oncology abstracts 2016–2018. It was expected that data presented earlier would be captured in full publications. Search terms included “second line”, “neuroendocrine carcinoma”, “neuro-endocrine carcinoma”, “platinum” (for full electronic search strategy, please see Supplementary Table S1), and was limited to English language articles.

**Study selection**

Inclusion criteria for the primary analysis were: studies reporting survival and/or response data for patients with EP-PD-NEC receiving second-line therapy. Studies were excluded if they were case reports or reviews, trials in progress, or if they only contained data on patients with lung primaries or mixed adenoneuroendocrine carcinoma (MANEC). Additionally, studies exploring treatment of well-differentiated grade 3 neuroendocrine tumours exclusively were excluded, as were those that did not contain individual data for patients with poorly differentiated NECs, or provided no data on actual second-line treatment or its outcomes. Selection pathological criteria for studies included in this systematic review and meta-analysis were based on the staging stated within individual method sections of publications and utilised over the period of time that associated patients were treated, and so may not align with the 2017 or 2019 World Health Organisation (WHO) classification of neuroendocrine neoplasms. Data were collected by two authors (MMN, TJ). The results were pooled, and all potentially relevant articles were retrieved in full; MMN assessed the full articles for eligibility. Duplicate publications were excluded. Disagreement was resolved by consensus.

**Data extraction**

Data were collected using predesigned electronic forms. The following details were extracted: name of first author, year of publication, total number
**Table 1.** Details of studies included in meta-analysis (second-line systemic treatment in patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma).

| Study                | Number of patients (n) | Study design | First-line therapy | Therapeutic agent (Second-line) | RR (%) | PFS (mo) | OS (mo) |
|----------------------|------------------------|--------------|--------------------|---------------------------------|---------|----------|---------|
| Apostolidis *et al.* 11 | 30                     | Retrospective | Platinum-based     | Topotecan                       | 7       | 2.1      | 4.1     |
| Araki *et al.* 12     | 19                     | Retrospective | Platinum-based     | Amrubicin                        | 19      | 3.8      | 7.7     |
| Chen *et al.* 13      | 11                     | Retrospective | Not available      | 5-Fluorouracil/Irinotecan (FOLFIRI) ± bevacizumab | 27      | 3.8      | 6.3     |
| Ebata *et al.* 14     | 13                     | Retrospective | Platinum-based     | Amrubicin                        | 45      | 6.0      | 10.6    |
| Frizziero *et al.* 15 | 16                     | Retrospective | Platinum-based (n=13), Streptozotocin/capecitabine (n=1), not available (n=2) | Carboplatin/Etoposide | 31 | 4.5 | 12.5 |
| Hadoux *et al.* 16    | 20                     | Retrospective | Platinum-based     | 5-Fluorouracil/Oxaliplatin (FOLFOX) | 29 | 4.5 | 9.9 |
| Hattori *et al.* 17   | 12                     | Retrospective | Platinum-based     | Amrubicin                        | 50      | 2.5      | 4.8     |
| Heetfeld *et al.* 8   | 79                     | Retrospective | Platinum/etoposide in 113 [68%] of entire population receiving first-line (n=167) | Multiple agents* | 16 | 3.0 | 7.6 |
| Hentic *et al.* 12    | 19                     | Retrospective | Platinum/etoposide | 5-Fluorouracil/Irinotecan (FOLFIRI) | 31 | 4.0 | 18.0 |
| Ichikawa *et al.* 18  | 13                     | Prospective [phase II single arm] | Platinum-based | Temozolomide | 15 | 1.7 | 6.3 |
| Kasahara *et al.* 19  | 18                     | Retrospective | Platinum-based     | Amrubicin                        | 11      | 4.0      | 9.1     |
| Okuyama *et al.* 20   | 23                     | Prospective [phase II single arm] | Platinum-based | Everolimus | 0 | 1.15 | 7.5 |
| Olsen *et al.* 21     | 28                     | Retrospective | Platinum-based     | Temozolomide                      | 0       | 2.4      | 3.5     |
| Olsen *et al.* 22     | 22                     | Retrospective | Carboplatin/etoposide | Topotecan | 0 | 2.1 | 3.2 |

(Continued)
| Study            | Number of patients (n) | Study design | First-line therapy | Therapeutic agent (Second-line) | RR (%) $^5$ (Second-line) | PFS (mo) (Second-line) | OS (mo) (Second-line) |
|------------------|------------------------|--------------|--------------------|---------------------------------|----------------------------|------------------------|------------------------|
| Sorbye et al. $^3$ | 100                    | Retrospective| Platinum/etoposide in 224 [89%] of entire population receiving first-line (n = 252) | Multiple agents** | 18 | NR | 19 |
| Welin et al. $^21$| 25                     | Retrospective| Cisplatin/etoposide (n = 24), Docetaxel/doxorubicin (n = 1) | Temozolomide ± Capecitabine | 33 | 6.0 | 22.0 |
| Yamaguchi et al. $^{10,***}$ | 25                  | Retrospective| Platinum-based in 206 [80%] of entire population receiving first-line (n = 258), gemcitabine-based (n = 10), fluoropyrimidine-based (n = 37) | Temozolomide ± Capecitabine | 33 | 6.0 | 22.0 |
| Yamaguchi et al. $^{10,***}$ | 23                  | Retrospective| Platinum-based in 206 [80%] of entire population receiving first-line (n = 258), gemcitabine-based (n = 10), fluoropyrimidine-based (n = 37) | Etoposide + Cisplatin or Carboplatin | 17 | 1.9 | 5.0 |
| Yamaguchi et al. $^{10,***}$ | 21                  | Retrospective| Platinum-based in 206 [80%] of entire population receiving first-line (n = 258), gemcitabine-based (n = 10), fluoropyrimidine-based (n = 37) | Irinotecan | 5 | 2.2 | 5.9 |
| Yamaguchi et al. $^{10,***}$ | 21                  | Retrospective| Platinum-based in 206 [80%] of entire population receiving first-line (n = 258), gemcitabine-based (n = 10), fluoropyrimidine-based (n = 37) | Tegafur (S-1) | 27 | 2.4 | 12.2 |
| Yamaguchi et al. $^{10,***}$ | 5                   | Retrospective| Platinum-based in 206 [80%] of entire population receiving first-line (n = 258), gemcitabine-based (n = 10), fluoropyrimidine-based (n = 37) | Irinotecan + Cisplatin | 40 | 4.8 | 8.7 |
| Yao et al. $^{24}$ | 21                    | Prospective (phase II) | Not available | Spartalizumab (PDR001) | 5 | NR | NR |
| Zhang et al. $^{25}$ | 28                    | Prospective (phase II) | Not available | Toripalimab (JS001) | 18 | NR | NR |

Mo, months; NR, not reported; OS, Overall survival; PFS, Progression-free survival; RR, response rate.

$^5$Response as per Response Evaluation Criteria In Solid Tumours (RECIST) 1.0 or 1.1.

*FOLFIRI, FOLFOX/Capecitabine/Oxaliplatin, Platinum/etoposide, temozolomide-based, clinical trials testing sunitinib, gemcitabine, docetaxel/cisplatin/5-Fluorouracil, dacarbazine-based, etoposide.

**Temozolomide-based (n = 35), Taxotere-based (n = 20), Not otherwise specified (n = 45).

***Data is presented for each individual regimen used within this study.
of patients included in the analysis, modality of data collection (prospective, retrospective), basic patient demographic data [including gender, age and Eastern Co-operative Oncology Group Performance Status (ECOG PS)], primary tumour site location, therapeutic regimen(s) used, proportion of patients with a Ki-67 >55%, or with small cell/large cell morphology, grade 3/4 adverse events (AEs): anaemia, neutropenia, thrombocytopenia. RRs were recorded according to Response Evaluation Criteria in Solid Tumours (RECIST) version 1.0/1.1, depending on year of study publication. Additionally, the following were extracted, where available: median progression free survival (PFS) and OS.

**Statistical methods and analyses**

Associations between baseline factors: age, gender, ECOG PS, primary tumour location, morphology, Ki-67 and second-line treatment characteristics (treatment received and grade 3/4 haematological toxicity), and RR, PFS and OS were assessed with a mixed effects meta-regression, weighted by individual study sample size, using the weighted least squares (mixed effect) function.31

Due to a small sample size, associations were reported quantitatively, based on magnitude of beta “β” coefficient rather than statistical significance.32 In recent years, the American Statistical Association has highlighted the limitations of basing decisions on p-values, emphasising that statistical significance can be the result of large effect size, high statistical power or a combination of the two. It was emphasised that the effect size can be more important than the p-value. In the case of our meta-regression, statistical power is derived from the number of studies and not the number of patients. As such, despite >500 patients included, statistical power in the regression model was very low. We therefore elected to report data in line with the recommendations of the American Statistical Association and focus on quantitative effects rather than just statistical significance.33,34

A $\beta$ coefficient of 0.32 is defined as significant, 0.45 as substantially significant and 0.60 as highly significant (0.60 considered clinically meaningful).32

**Results**

Of 90 studies identified initially, 19 were eligible (patients included from September 1996 to March 2018), including 4 prospective and 15 retrospective studies (Figure 1).8–25,35 There were no published results from second-line randomised studies in advanced EP-PD-NEC reported in the literature. One of the identified excluded publications included combined data from 76 patients from two single-arm phase II trials of oxaliplatin-fluoropyrimidine chemotherapy plus bevacizumab in patients with advanced well-, moderately and poorly differentiated extra-pulmonary tumours where prior chemotherapy, excluding oxaliplatin, was allowed.36 In this publication, a total of six patients with EP-PD-NEC were included, and in one of the phase II studies, the poorly differentiated cohort closed to recruitment due to poor accrual. This study did not meet eligibility for inclusion in this meta-analysis since no individual survival data or indication of line of therapy for those with EP-PD-NEC was reported.36

The choice of first-line chemotherapy was predominantly platinum-based (Table 1). In total, 15 different regimens were used in second-line; monotherapy in 284 patients (48%), and combination in 119 patients (20%), whereas the remaining 179 patients (31%) were included in two studies investigating multiple regimens,8,9 and it was not possible to deduce the exact number who received monotherapy or combination regimens (see Table 1 for details).

Analysis comprised 582 patients, with a median number of 19 patients in each study (range 5–100). The median age was 59 years (range 53–69). A total of 15 studies reported on gender ($n$= 302; with 62% being male), with 11 studies reporting ECOG PS for 235 patients; ECOG PS 0–1: 85%, ECOG PS 2–3: 15%.

A total of 13 studies reported on primary tumour site location ($n$= 252); unknown primary: 32%, pancreas: 29%, oesophagus/gastro-oesophageal junction: 15%, colorectal: 9%, stomach: 6%, liver/biliary primary: 4%, prostate/bladder/ureter: 4%, small bowel: 1%, and 4 studies ($n= 84$) reported on the proportion of patients with NECs with a Ki-67 >55%,11,13,17,18 Six studies reported on morphology ($n= 134$): large cell: 42%, small cell: 37%, otherwise not specified: 21%.11,13,16,21,31,32

Median RR was 18% (range 0–50; 0% for single-agent everolimus, temozolomide, topotecan; 50% with amrubicin; Table 1). Median PFS was 2.5 months (range 1.2–6.0) and median OS was 7.6 months (range 3.2–22) (Table 1).
Studies with a higher proportion of patients with a liver/biliary primary had higher RRs ($\beta = 0.65$), and those with a higher proportion of patients with a Ki-67 $> 55\%$ had a lower RR ($\beta = -0.73$) and shorter OS ($\beta = -0.82$). No associations with PFS were quantitatively significant (Table 2–4). The $\beta$ for association of combination therapy (versus monotherapy) with RR, PFS and OS were not quantitatively significant ($<0.60$), but had statistically significant $p$ values ($p = 0.008$, 0.012 and 0.019 respectively) (Tables 2–4).

**Discussion**

As there are limited large-scale published studies in the literature regarding EP-PD-NECs, and the data available are predominantly from single institutions, limited by size and/or sites evaluated, a comparative population-based study of lung and EP-PD-NECs from the Surveillance, Epidemiology, and End Results (SEER) programme between 1973 and 2012, was undertaken in 2018. This provides additional information to the neuroendocrine community on this disease group. Of 162,983 cases of NEC identified (all stages), 8.7% were EP-PD-NECs, and of these, approximately 37% were gastrointestinal, 28% were of unknown primary and 34% of other sites. Lung NEC had the greatest percentage of small cell morphology (95.2%), and gastrointestinal NEC had the least (38.7%). Unfortunately, the use of systemic chemotherapy was not noted within the SEER database and could not be explored further.
This current systematic review and meta-analysis aimed to address some of the unanswered questions related to the demographics, response and survival for patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma, receiving second-line systemic therapy, and included data from predominantly small retrospective studies, with a lower median age of patients (59 years) than the SEER study (67 years),\(^\text{39}\) predominantly with a better ECOG PS (0–1), possibly reflecting a population fit enough to receive second-line therapy. There were a greater proportion of males in the current systematic meta-analysis (63%) in comparison to the SEER database (51%).\(^\text{39}\)

However, despite the size limitation of this current meta-analysis, similar morphology proportions to

| Table 2. Meta-regression* association of variables with response rate in meta-analysis of second-line systemic treatment in patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma. |
|-------------------|-------|-------|
| Variable | \(\beta\) | \(p\) |
| Median age | -0.341 | 0.21 |
| Proportion male | -0.278 | 0.30 |
| Proportion ECOG PS 0-1 | -0.214 | 0.50 |
| Proportion primary stomach | -0.142 | 0.66 |
| Proportion primary oesophagus | -0.109 | 0.74 |
| Proportion primary gastro-oesophageal junction | 0.180 | 0.58 |
| Proportion primary upper gastrointestinal (includes all 3 of the above) | 0.037 | 0.91 |
| Proportion primary colorectal | -0.082 | 0.80 |
| Proportion primary small bowel | 0.037 | 0.91 |
| Proportion primary liver | 0.653 | 0.11 |
| Proportion primary pancreas | -0.225 | 0.44 |
| Proportion hepatopancreaticobiliary (includes 2 of the above (liver + pancreas)] | 0.034 | 0.94 |
| Proportion primary GU (includes prostate and bladder) | 0.287 | 0.37 |
| Proportion Grade 3–4 anemia | 0.193 | 0.51 |
| Proportion Grade 3–4 neutropenia | 0.343 | 0.28 |
| Proportion Grade 3–4 thrombocytopenia | -0.346 | 0.27 |
| Median Ki-67 | -0.295 | 0.57 |
| Proportion with Ki-67 >55% | -0.728 | 0.27 |
| Proportion large cell | -0.084 | 0.88 |
| Proportion small cell | 0.297 | 0.57 |
| Combination therapy (reference: monotherapy) | 0.550 | 0.008 |
| Other therapy** (reference: monotherapy) | 0.253 | 0.34 |

ECOG PS, Eastern Co-operative Oncology Group Performance Status, GU, Genitourinary.

*Meta-regression (linear regression weighted by individual study sample size, using the weighted least squares [mixed effect] function.\(^\text{31}\)

**FOLFIRI, FOLFOX/Capecitabine/Oxaliplatin, Platinum/etoposide, temozolomide-based, clinical trials testing sunitinib, gemcitabine, docetaxel/cisplatin/S-Fluorouracil, dacarbazine-based, etoposide, docetaxel-based and not otherwise specified.
the SEER database study were reported, although there was a lower proportion of GEP NECs and a higher proportion of genitourinary NECs in the SEER database (within the EP-PD-NEC subgroup), but will add to the limited published literature in the second-line advanced setting for EP-PD-NEC. In approximately one-fifth of cases in this meta-analysis, where morphology was reported, an accurate classification was not specified, which has also been demonstrated previously, and requires addressing in guidelines and prospective studies.

This meta-analysis also reported that the variety of regimens used was diverse, including mono- and combination therapies, using topoisomerase I and II inhibitors, antimetabolites, alkylating agents, as

| Variable                                      | β     | p     |
|-----------------------------------------------|-------|-------|
| Median age                                    | −0.430| 0.13  |
| Proportion male                               | −0.318| 0.25  |
| Proportion ECOG PS 0-1                        | −0.066| 0.85  |
| Proportion primary stomach                    | −0.027| 0.94  |
| Proportion primary oesophagus                 | 0.040 | 0.90  |
| Proportion primary gastro-oesophageal junction| −0.225| 0.48  |
| Proportion primary upper gastrointestinal     | −0.164| 0.61  |
| Proportion primary colorectal                 | 0.253 | 0.43  |
| Proportion primary small bowel                | 0.075 | 0.82  |
| Proportion primary liver                      | 0.137 | 0.77  |
| Proportion primary pancreas                   | −0.184| 0.53  |
| Proportion hepatopancreaticobiliary           | −0.073| 0.88  |
| Proportion primary GU (includes prostate and  | 0.238 | 0.46  |
| bladder)                                      |       |       |
| Proportion Grade 3-4 anemia                   | −0.177| 0.56  |
| Proportion Grade 3-4 neutropenia              | 0.071 | 0.83  |
| Proportion Grade 3-4 thrombocytopenia         | −0.344| 0.27  |
| Median Ki-67                                  | −0.353| 0.49  |
| Proportion with Ki-67 >55%                    | −0.514| 0.49  |
| Proportion large cell                         | −0.024| 0.96  |
| Proportion small cell                         | −0.307| 0.55  |
| Combination therapy [reference: monotherapy]  | 0.549 | 0.012 |
| Other therapy** [reference: monotherapy]      | 0.195 | 0.50  |

ECOG PS, Eastern Co-operative Oncology Group Performance Status; GU, Genitourinary; PFS, progression-free survival.

*Meta-regression (linear regression weighted by individual study sample size, using the weighted least squares (mixed effect) function.**FOLFIRI, FOLFOX/Capecitabine/Oxaliplatin, Platinum/etoposide, temozolomide-based, clinical trials testing sunitinib, gemcitabine, docetaxel/cisplatin/5-Fluorouracil, dacarbazine-based, etoposide, docetaxel-based and not otherwise specified.
well as targeted therapy such as a mammalian target of rapamycin inhibitor (everolimus), a humanised murine monoclonal antibody targeting the vascular endothelial growth factor (VEGF) ligand (bevacizumab) and anti-programmed death 1 agents (spartalizumab and toripalimab). Although quantitatively not highly significant, the significant $p$ values correlating with the use of combination therapy versus monotherapy and their association with RR, PFS and OS indicate consistent benefit across studies, but may also be a reflection of the population of patients fit enough to receive the former treatment.

An additional study was published following completion of the literature review for this Table 4. Meta-regression* association of variables with OS in meta-analysis of second-line treatment in patients with advanced extra-pulmonary poorly differentiated neuroendocrine carcinoma.

| Variable                                        | $\beta$ | $p$ |
|------------------------------------------------|--------|-----|
| Median age                                      | 0.571  | 0.04|
| Proportion male                                 | 0.445  | 0.11|
| Proportion ECOG PS 0-1                          | -0.102 | 0.78|
| Proportion primary stomach                      | -0.099 | 0.76|
| Proportion primary oesophagus                   | -0.184 | 0.57|
| Proportion primary gastro-oesophageal junction  | -0.304 | 0.34|
| Proportion primary upper gastrointestinal [includes all 3 of the above] | -0.365 | 0.24|
| Proportion primary colorectal                    | 0.184  | 0.57|
| Proportion primary small bowel                  | -0.191 | 0.55|
| Proportion primary liver                        | 0.260  | 0.57|
| Proportion primary pancreas                     | 0.283  | 0.35|
| Proportion hepatopancreaticbiliary [includes 2 of the above (liver + pancreas)] | 0.369 | 0.42|
| Proportion primary GU [includes prostate and bladder] | -0.059 | 0.86|
| Proportion Grade 3–4 anemia                     | -0.469 | 0.12|
| Proportion Grade 3–4 neutropenia                | -0.308 | 0.33|
| Proportion Grade 3–4 thrombocytopenia           | -0.378 | 0.23|
| Median Ki-67                                     | -0.478 | 0.34|
| Proportion with Ki-67 >55%                      | -0.819 | 0.18|
| Proportion large cell                           | -0.529 | 0.36|
| Proportion small cell                           | 0.144  | 0.82|
| Combination therapy [reference: monotherapy]    | 0.520  | 0.019|
| Other therapy** [reference: monotherapy]        | 0.226  | 0.46|

ECOG PS, Eastern Cooperative Oncology Group Performance Status; GU, Genitourinary; OS, overall survival.

*Meta-regression (linear regression weighted by individual study sample size, using the weighted least squares [mixed effect] function).

**FOLFIRI, FOLFOX/Capecitabine/Oxaliplatin, Platinum/etoposide, temozolomide-based, clinical trials testing sunitinib, gemcitabine, docetaxel/cisplatin/5-Fluorouracil, dacarbazine-based, etoposide, docetaxel-based and not otherwise specified.
| Therapeutic agents | Primary objective | Key eligibility criteria | Planned recruitment (n) | Recruiting location | Status | ClinicalTrials.gov identifier |
|--------------------|-------------------|--------------------------|-------------------------|--------------------|--------|-----------------------------|
| Capecitabine and temozolomide or 5-fluorouracil (5-FU)/folinic acid/irinotecan (FOLFIRI) | Histological diagnosis of gastroenteropancreatic and lung NEC, grade 3, Ki-67 > 20%, received previous platinum-based treatment in first-line advanced setting. | 112 | Italy | Recruiting | NCT03387592 | Disease Control Rate (% of patients achieving complete, partial response and stable disease) lasting at least 12 weeks (RECIST 1.1) and incidence of treatment-related AEs. |
| FOLFIRI ± Bevacizumab | Poorly differentiated NEC of gastrointestinal tract or unknown primary, received previous platinum/etoposide-based therapy in first-line advanced setting. | 45 | France | Recruiting | NCT0280857 | Proportion of patients alive at 6 months. |
| Liposomal irinotecan/5-FU/low-dose etoposide | Poorly differentiated NEC, grade 3, Ki-67 > 20%, received prior first-line platinum-based chemotherapy in advanced setting. | 102 | United Kingdom | Recruiting | NCT03837977 | 6-month PFS rate. |
| Everolimus | Pathologically-confirmed poorly differentiated NEC, who received previous platinum/etoposide-based chemotherapy. | 40 | Germany | Recruiting | NCT02113800 | Incidence of AEs. |
| Lipotecan (TLC388) | Poorly differentiated NEC, grade 3, or well or moderately differentiated NEC that switched to grade 3, or neuroendocrine carcinoma of unknown primary, allowed if lung primary excluded, grade 3, Ki-67 > 20%, received prior platinum-based chemotherapy in first-line advanced setting. | 44 | Taiwan | Completed | NCT02457273 | Objective RR. |

AE, adverse event; NEC, neuroendocrine carcinoma; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumours; RR, response rate.

A http://www.ClinicalTrials.gov search was last updated on 26 May 2019.
meta-analysis,\textsuperscript{41} which reported the efficacy and safety of the monoclonal antibody against VEGFR2, ramucirumab, combined with chemotherapy in patients with pre-treated metastatic gastric NEC. A total of 17 patients received ramucirumab plus paclitaxel ($n=13$), ramucirumab plus irinotecan ($n=2$), or ramucirumab monotherapy ($n=2$), with an overall encouraging RR, PFS and OS of $59\%$, 7.7 months and 16.1 months respectively, versus $8\%$, 1.8 months and 8.6 months in those receiving chemotherapy alone ($n=13$; amrubicin: $n=6$, irinotecan: $n=4$, paclitaxel: $n=3$). The authors concluded that the ramucirumab/chemotherapy combination demonstrated promising activity, without severe or unexpected safety issues, and may be due to higher VEGFR2 expression in gastric NEC.\textsuperscript{41}

Second-line therapy for patients with advanced EP-PD-NEC had limited efficacy in this meta-analysis, and a high Ki-67 was associated with treatment outcomes, as reported previously.\textsuperscript{9,42} Indeed, the relevance of the proliferation marker Ki-67 in neuroendocrine tumours has long been reflected in the classification system,\textsuperscript{43} and is also known to be prognostic in other tumour sites, such as breast cancer.\textsuperscript{44} In this current meta-analysis, the finding of a lower RR in studies with a higher proportion of patients with Ki-67 $>55\%$, seems in contrast with that reported in the NORDIC NEC study,\textsuperscript{9} but the majority of these patients receiving second-line treatment will have developed resistance to first-line platinum-based chemotherapy and the Ki-67 may be a predictive factor of response to platinum, in addition to being a negative prognostic factor.

This meta-analysis also indicated that studies with a higher proportion of patients with a liver/biliary primary had a higher RR,\textsuperscript{17,18,35} but it may be that these were actually metastases rather than primaries, and further inferences cannot be made. It should also be noted that the number of patients with a liver/biliary primary included was small, and so large prospective studies are required to evaluate this finding further.

To address the lack of a standard-of-care second-line therapy in this disease group, there are some on-going clinical trials in this setting reported on clinicaltrials.gov, which may guide future treatment decisions (Table 5).\textsuperscript{45,46} One of these trials reported interim data at the Annual American Society of Clinical Oncology conference this year [ClinicalTrials.gov identifier: NCT02457273].\textsuperscript{47} In 20 evaluable patients who received TLC388 (lipotecan hydrochloride, a novel derivative of topotecan hydrochloride) as second-line treatment in a single-arm phase II trial in patients with advanced PD-NEC, including lung, there were no responses reported, disappointingly, and the median PFS and OS were 1.8 and 4.3 months, respectively.\textsuperscript{47}

Limitations of the current manuscript include that this was a literature-based meta-analysis, with associated publication bias, with inference from study-level data in meta-regression rather than individual patient data, and with other limitations relating to the methodological rigour of included studies. The studies analysed were predominantly from single institutions and retrospective, and pathological classification cannot be confirmed, nor alignment with the 2017 and 2019 WHO pathological classification for neuroendocrine neoplasms, with the added associated inherent bias, such as lack of a control arm in a randomised study population. However, this study does include a relatively large number of patients in an understudied disease group of unmet need, and thus may help inform future study design.

Prospective randomised studies are warranted to enable exploration of new treatment strategies, and this current meta-analysis provides a reference benchmark. Despite the low incidence and aggressive nature of these malignancies, multi-institutional collaborative efforts will ensure adequate recruitment to prospective clinical trials, preferably randomised, to deliver more evidence-based guidance, with the potential for associated translational-rich research to improve outcomes for these patients.

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ORCID iD
Mairead G. McNamara https://orcid.org/0000-0002-2272-3678

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