EXPERT REVIEWS

Neuroendocrine neoplasms of the GI tract: the role of cytotoxic chemotherapy

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Neuroendocrine neoplasms (NENs) comprise a heterogeneous group of neoplasms derived from peptide- and amine-secreting cells of the neuroendocrine system. NENs commonly arise in the GI tract but can arise in most organs of the body. NENs in different organs share many common pathologic features. Although the incidence of NENs is not high, the prevalence is not low because many patients may live relatively long periods without major symptoms from the disease. While many of these tumors lead an indolent clinical course, they constitute a therapeutic challenge when they progress, metastasize and become symptomatic. Treatment requires a multidisciplinary approach including cytotoxic chemotherapy. Almost all clinical trials investigating cytotoxic chemotherapy in NENs are small single-arm studies and guidelines are derived from expert opinion and from extrapolating results from small cell lung cancer studies. This article briefly reviews NENs before focusing on reviewing data on the role of cytotoxic chemotherapy studies in NENs.

Keywords: cytotoxic chemotherapy • neuroendocrine carcinoma • neuroendocrine neoplasms • neuroendocrine tumors

Neuroendocrine tumors or neuroendocrine neoplasms (NENs), defined as epithelial neoplasms with predominantly neuroendocrine differentiation, arise in most organs of the body and share many common pathologic features. They comprise a heterogeneous group of neoplasms derived from peptide- and amine-secreting cells of the neuroendocrine system. NENs commonly arise from the GI tract (~66%), with the next most common being bronchopulmonary NENs (31%), but NENs can also arise in other organs, including the ovaries and testes [1]. Some clinical and pathologic features of these tumors are characteristic of the organ of origin. However, NENs share other attributes irrespective of their anatomic site. Well-differentiated NENs (low and intermediate grade) and poorly differentiated (high-grade) neuroendocrine carcinomas (NECs) have similar neuroendocrine marker expression. They are characterized by the intracellular presence of endocrine tissue markers, such as chromogranin A (CGA), synaptophysin and neuron-specific enolase. Despite this, well-differentiated and poorly differentiated tumors are genetically and clinically distinct.

Well-differentiated NENs arise from the diffuse neuroendocrine system. Some may secrete neuropeptides, causing a range of clinical syndromes – most classically, carcinoid syndrome. Nevertheless, many are clinically silent and, in others, the nonspecificity of the associated symptoms leads to delayed diagnosis. Consequently, many tumors present only after metastasis has occurred. Despite this, the clinical courses of these diseases vary; patients frequently have tumors that are indolent and progress slowly over several years. By contrast, poorly differentiated NENs are highly aggressive and are often managed by chemotherapy.

Diagnosis & staging of NENs

Histopathologic classification of NENs

In the past, NENs were thought to be a homogenous group of tumors and were all treated in a similar manner. However, over the last decade, a significant amount of research has identified NENs as being a heterogeneous group of tumors with varying biological activity depending on the tumor site of origin and cellular characteristics.

The diagnosis of a gastroenteropancreatic (GEP)-NEN is made based on histological

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examination including morphology and immunoperoxidase stains. Morphologically, these neoplasms typically present as 'salt and pepper' nuclei, with low mitotic rate and uniformity in shape and size. By contrast, poorly differentiated carcinomas have less recognizable features. The most common immunoperoxidase stains that are performed include synaptophysin and CGA. Other markers include cluster of differentiation (CD), CD56, CD57 and neural cell adhesion molecules. Further immunostains can also help to ascertain the site of the primary tumor. Thyroid transcription factor 1 for lung and/or thyroid primary, caudal type homeobox 2 for intestinal primary, pancreatic and duodenal homeobox 1 for pancreas [2], neuroendocrine secretory protein 55 for pancreatic endocrine tumors [3]. Cytokeratin 7 and CK20 suggest a gastrointestinal (GI) tumor and exclude adrenal cortical carcinoma, germ cell tumor, prostate carcinoma, renal cell carcinoma and hepatocellular carcinoma [4].

Tumor grade and differentiation are used to categorize the neoplasms. 'Tumor differentiation' refers to the extent of resemblance to the normal cellular counterpart, whereas 'tumor grade', which is related to differentiation, refers to the degree of biological aggressiveness. Histopathologic features, for example, degree of cell architecture, such as small versus large, are used. The Ki-67 proliferation index (%) and mitotic index have been shown to have prognostic significance and should be used routinely. The Ki-67 index (%) correlates well with size, tumor behavior and proliferation.

A number of different systems exist to classify and better define these tumors, including those from the European Neuroendocrine Tumor Society, WHO and International Union Against Cancer. However, the 2010 WHO classification has reached a consensus in defining NENs [5,6].

According to the 2010 WHO system, grading provides classification for grades 1, 2 and 3, corresponding to low, intermediate or high (Table 1). Classification for NENs thus, separates well-differentiated tumors into grade (G)1, G2 and G3, whereby G is synonymous with the proliferation index as measured by Ki-67 index (%). In this classification, G1 is low grade (Ki-67: <2%), G2 is intermediate grade (Ki-67: 2–20%) and all poorly differentiated NENs are G3 or high-grade (Ki-67: >20%) NECs. The latter are more likely to be treated with cytotoxic chemotherapy, which is the focus of this review.

Biochemical markers/secretory products of NENs

NENs are either functioning (hormone secreting) or nonfunctioning (nonsecreting). Functioning NENs can have a variety of measurable proteins, including serotonin and its breakdown product 5-hydroxyindoleacetic acid that can be measured in the urine. NENs could secrete specific markers, such as gastrin for gastrinoma or insulin for insulinoma.

General markers include CGA, which is a soluble secretory glycoprotein normally contained in neuroendocrine cell vesicles. Plasma assays for intact CGA and cleavage products released by specific NENs are available and used routinely. It is elevated in 60–100% of patients with NENs, including in functioning and nonfunctioning midgut carcinoids, pancreatic islet tumors and pheochromocytomas. However, chronic atrophic gastritis, renal failure, liver impairment, proton-pump inhibitors and inflammatory bowel disease can lead to a false-positive result [2].

These markers are useful in low- and intermediate-grade NENs but not in high-grade NECs. Another general marker is neuron-specific enolase which may be elevated in high-grade tumors and may be associated with survival; however, its clinical value still needs to be elucidated [7].

Imaging of NENs

Different imaging modalities are employed for staging of NENs, including anatomical and functional imaging. To detect sites of disease with anatomical imaging, multislice computed tomography (CT) and MRI are the most widely used methods. CT enteroclysis has become the standard for diagnosing primary small bowel tumors. Multislice CT remains the standard for detecting liver lesions. However, for indeterminate lesions, dynamic contract-enhanced ultrasound and dynamic contract-enhanced-ultrasound-MRI can be used. Upper GI endoscopy combined with endoscopic ultrasound is increasingly used for local staging of small pancreatic lesions, particularly those located in the head and body, and lesions in the duodenal wall. Double balloon enteroscopy and capsule endoscopy may also be used when appropriate.

At least 80% of GEP-NENs express the somatostatin receptor subclasses 2 and 5; therefore, functional imaging with radiolabeled somatostatin analogues (SSAs), which bind with high affinity to these receptors, is usually used as part of the diagnostic process. It is also understood that the degree of uptake can be used to guide treatment and response.

Indium-111 pentetreotide (octreotide scan) is used for the detection of suspected GEP-NENs and the exclusion of distant metastasis for patients who have surgically resectable disease. The octreotide scan is expected to be replaced

Table 1. Grading system of neuroendocrine neoplasms.

Histological classification	Well differentiated (low grade, G1)	Moderately differentiated (intermediate grade, G2)	Poorly differentiated (high grade, G3)
Prognosis	Prolonged survival	Intermediate	Poor
Ki-67 index (%)	≤2	2–20	>20
Mitotic count (10 HPF)	<2	2–20	>20
Necrosis	Absent		Present

G: Grade; HPF: High-power field.

by more sensitive methods, including PET/CT, which can be performed using a number of tracers. Used tracers include ^{18}F-fluorodeoxyglucose (FDG), ^{68}Ga-pentetreotide (or other SSAs such as [yttrium-90-DOTA-Tyr 3]-octreotide (DOTATOC) and [177 Lu-DOTA-Tyr 3-Thr 8]-octreotide (DOTATATE), ^{18}F-dihydrooxyphenylalanine and ^{11}C-5-hydroxytryptophan. FDG-PET is most useful in the setting of poorly differentiated NEC with a high percentage of Ki-67 index (%), as these tumors have lost somatostatin receptor expression. Not surprisingly, in one study survival times for FDG-negative patients were significantly longer than those for FDG-positive patients [8]. FDG-PET is not recommended in well-differentiated tumors in which PET scanning with ^{68}Ga-octreotide has much higher sensitivity and specificity in detecting disease compared with indium-111 octreotide [9]. Given the current limited widespread availability of novel techniques, scintigraphy with somatostatin analogues is still used.

Multiple imaging modalities may be needed to detect small, biochemically diagnosed tumors. All modalities can frequently fail to notice small liver metastases (i.e., tumors <0–5 mm in diameter), underestimating the true disease extent.

Treatment of NENs

Treatment requires a multidisciplinary approach and often the combination of several therapy modalities. Recently, there has been increasing interest in NENs, partly because detection of asymptomatic tumors is more common with improving imaging sensitivity.

Radical surgery is often curative in low and intermediate tumors. Resection of primary and metastatic disease should be considered if imaging suggests that all the disease is resectable. Debulking surgery can be considered for control of symptoms that are resistant to medical management in metastatic disease.

Locoregional therapies are often employed, especially in the case of liver-dominant metastatic NENs whereby transarterial embolization can be delivered to the tumor. This can be either bland embolization or include chemotherapy embolization. Radioactive treatment with SIR-Spheres® (Sirtex Medical Limited, Lane Cove, Australia) which are radioactive yttrium 90 microspheres (SIR-Spheres) is also employed to target liver metastases.

The term 'biotherapy', often used in NEN literature, refers to the use of IFN-α and SSAs, for example, octreotide, alone or in combination. Use of this therapy may affect cell proliferation control, angiogenetic processes, hormone release and clinical symptoms. Several studies have demonstrated that IFN-α has significant activity in low-grade NEN [10]. Using interferon may lead to biochemical and subjective response rates of approximately 50% and stabilization of disease in 35% of patients, on average. The median duration of response is 30 months [11]. SSAs are better tolerated than interferon and combining the drugs may achieve a better control of 'carcinoid' symptoms [12]. Mainly uncontrolled trials of SSAs have included a mixture of GEP-NEN primary sites [10]. SSAs may also offer a survival benefit, as shown in the randomized but relatively small (n = 85) placebo-controlled prospective randomized study on the antiproliferative efficacy of octreotide long-acting octreotide in patients with metastatic neuroendocrine midgut tumors (PROMID) study [13].

Peptide-receptor radionuclide therapy (PRRT) using SSAs or radioactive octreotide-labeled treatment is another modality that can be used with these patients using different methods and strategies. PRRT involves targeting of a molecule-radionuclide conjugate to specific surface receptors on tumor cells. Upon binding to the receptor, the radioisotope-molecule complex is endocytosed. This technique is thus selective for tumor and peritumoral cells, with relative sparing of intervening non-neoplastic tissue, although there is a degree of bone marrow, renal and bladder (during elimination) exposure. Somatostatin receptor-expressing tumor cells can be targeted using yttrium-90, lutetium-177 or indium-111 radionuclides linked to an SSA.

Targeted systemic treatment is also showing promise in NENs, with positive results from Phase III studies using targeted therapies, mainly for low-grade and intermediate-grade pancreatic NENs [14,15]. These therapies include sunitinib, which is a tyrosine kinase inhibitor and everolimus, which inhibits the mTOR. Unfortunately, these agents have not been reported efficacious in high-grade tumors. The benefit was mostly in G1 and G2 pancreatic NENs. Most ongoing clinical trials investigating the role of molecular-targeted therapies in low- and intermediate-grade NENs generally exclude enrollment of high-grade tumors. Several clinical trials are currently ongoing [101], investigating numerous combinations of molecular-targeted therapies with cytotoxic chemotherapy. Detailed discussion of the modalities mentioned is beyond the scope of this article and there is recent literature that discusses these methods in detail [5,7,9,11].

Search strategy & selection criteria

Research for this review was identified by searches of the ISI Web of Science and PubMed (from 1970 to June 2012) databases. Medline medical subject headings used were 'cytotoxic', 'chemotherapy', 'carcinoid tumor', 'neuroendocrine tumor', 'neuroendocrine carcinoma' and 'clinical trial', combined with various keywords limiting the search to neuroendocrine tumors and clinical trials. The final search was conducted on 12 June 2012. Searches in PubMed were limited to clinical trials, meta-analyses, randomized controlled trials and systematic reviews (published in English). Retrospective case series were noted, but because of the known shortcomings of retrospective studies, results and conclusions are not discussed in as much detail in this review. Studies of bronchopulmonary NENs or non-GI sites were not included. Reference lists of retrieved articles were then searched to identify other relevant publications. In addition, the 'related articles' feature of PubMed was used to identify other relevant publications.

The Conference Proceedings Citation Index of the Web of Science database was searched to retrieve abstracts from relevant conferences, including the annual meetings of the American Society of Clinical Oncology up to the meeting of June 2012 and the European Neuroendocrine Tumor Society up to their 2012 meetings.

NENs is the terminology that encompasses neuroendocrine tumors and also neuroendocrine carcinomas, both covered in the article and hence the authors have used NENs to cover both.

Cytotoxic chemotherapy for NENs

Traditionally, chemotherapy has not been the mainstay of treatment for low- and intermediate-grade NENs. However, although high-grade NENs show a relatively high response rate to chemotherapy, high-grade tumors tend to relapse after initial response and have a worse prognosis. Chemotherapy is mainly used in patients with progressive and metastatic pancreatic NENs after failure of other treatment modalities such as SSAs (e.g., octreotide). Much of the literature has focused on the surgical and local treatment options for NENs but relatively few studies have focused on chemotherapy, which is an important treatment modality in both low- and high-grade NEC. Here, the authors review the literature on the use of chemotherapy in well-differentiated and poorly differentiated GEP-NENs.

Cytotoxic chemotherapies for NENs

Streptozocin-based chemotherapy

The earliest report of the activity of chemotherapy in NENs came from a patient who was treated with streptozocin (STZ) for a pancreatic islet-cell tumor in 1968 [16]. Pancreatic NENs appeared to have better response rate to chemotherapy compared with nonpancreatic GI tract NENs (Table 2).

In 1979, Moertel *et al.* reported on a study randomizing metastatic 'carcinoid' patients to either STZ plus 5-fluorouracil (5-FU) or STZ in combination with cyclophosphamide. The response rates noted were 33 and 26%, respectively. The study reports that they randomized 118 patients but it was only possible to evaluate 89 patients. However, in the results they describe 92 patients including 56 GEP-NENs, 17 bronchopulmonary NEN, 18 from unknown NEN of unknown primary and one ovarian NEN. Response assessment was either radiological or based on 30% reduction in size of clinically assessed enlargement of the liver [17].

Subsequently, in 1980, Moertel *et al.* reported on a study of 94 patients with advanced islet-cell carcinoma [18]. Patients were assigned to STZ alone or STZ plus 5-FU given in 5-day courses. The combination had advantages over STZ alone, with an overall response rate of 63 versus 36% and a complete response rate of 33 versus 12%. The median duration of response was 17 months. Response was assessed either radiologically or by the clinician assessing reduction in the size of hepatomegaly. This approach to response assessment would not be considered acceptable by current clinical standards. Nevertheless, based on the results of this trial, the combination of STZ and 5-FU became the standard therapy for advanced pancreatic NENs during the 1980s and early 1990s.

Over 10 years later, in 1992, Moertel *et al.* showed that STZ plus doxorubicin (DOX) had a combined biochemical and radiologic response rate of 69 versus 45% (p = 0.05) and a median survival of 26 versus 14 months (p = 0.004) [19]. Although STZ-based chemotherapy offers a reasonable response rate, it is also associated with significant side effects including severe nausea, vomiting and nephrotoxicity. DOX can also cause cardiac failure. Again, this study is likely to have overestimated objective response (OR) rates because response criteria included regression of clinical hepatomegaly as well as a 50% decrease of tumor markers. To date, STZ remains the only cytotoxic agent approved by the US FDA specifically for NENs.

In contrast to pancreatic NENs, other GI NENs are less sensitive to chemotherapy (Table 3). A Phase II and III study of 5-FU plus STZ or DOX alone in 172 chemo-naive patients with measurable progressive carcinoid tumor reported response rates of 22% for 5-FU plus STZ and 21% for DOX. The median response duration and median survival were 31 and 64 weeks, respectively, for the combination therapy and 26 and 48 weeks, respectively, for DOX. Thirty three patients who failed 5-FU plus STZ crossed over to DOX and achieved a response rate of 18%. Of the 35 patients who failed on DOX, 29% responded to 5-FU plus STZ. Hematologic toxicity was similar for both treatments; however, the 5-FU plus STZ patients experienced more vomiting but acceptable renal toxicity [20].

In 2005, Sun *et al.* compared STZ in combination with 5-FU with DOX/5-FU in a similar population. A response rate of 16% was achieved in both arms; however, median overall survival was significantly longer (24 vs 16 months) in the STZ/5-FU arm [21]. Sun *et al.* found no difference in response rates and progression-free survival (PFS) when 249 patients with advanced carcinoid tumors were randomized to either DOX with fluorouracil (FU/DOX) or STZ with fluorouracil (FU/STZ). FU/STZ (24.3 months) was superior to FU/DOX (15.7 months; p = 0.0267) in median survival. Hematologic toxicities were the major treatment-related toxicities for both FU/DOX and FU/STZ, and mild-to-moderate renal toxicity was reported in 40 (34.8%) out of 115 patients in the FU/STZ arm [21].

Table 2. Streptozocin for pancreatic neuroendocrine neoplasms.

Study (year)	Regimen	n	Response (%)	Survival (months)	p-value	Ref.
Moertel *et al.* (1980)	STZ	42	36	16.4	NS	[18]
	5-FU/STZ	42	63	26		
Moertel *et al.* (1992)	Chlorozotocin	33	30	18	<0.03	[19]
	5-FU/STZ	34	45	17	<0.04	
	DOX/STZ	38	69	26		

5-FU: 5-fluorouracil; DOX: Doxorubicin; NS: Not significant; STZ: Streptozocin.

Table 3. Streptozocin for nonpancreatic neuroendocrine neoplasms.

Study (year)	Regimen	Individuals (n)	Response (%)	Survival (months)	p-value	Ref.
Moertel *et al.* (1979)	STZ/5-FU	44	33	12.5	NS	[17]
	STZ/ CPM	48	26	11.5		
Engstrom *et al.* (1984)	DOX	81	21	11	0.250	[20]
	5-FU/STZ	80	22	15		
Sun *et al.* (2005)	5-FU/STZ	78	16	24	0.027	[21]
	5-FU/DOX	85	16	16		

5-FU: 5-fluorouracil; CPM: Cyclophosphamide; NS: Not significant; DOX: Doxorubicin; STZ: Streptozocin.

A more recent case series of metastatic or locally advanced NENs by Turner *et al.* reported an overall response rate of 33% (n = 79). This was 38% for pancreatic primary sites and 25% for nonpancreatic primary sites. The regimen used was a combination of 5-FU (500 mg/m^2), cisplatin (70 mg/m^2) and STZ (1000 mg/m^2) three-times weekly for up to six cycles. The authors reported that the regimen was effective with an acceptable toxicity profile [22]. However, this was a retrospective series with strong potential for selection bias.

Platinum combinations

Most guidelines recommend the combination of a platinum compound with etoposide for high-grade NENs. This combination is an established standard of care for small cell lung cancer that also has neuroendocrine differentiation. However, the data are less clear for nonpulmonary NENs and NECs.

In one study that examined the use of cisplatin and etoposide in high-grade NECs, 45 patients with metastatic NENs were treated with etoposide 130 mg/m^2 per day for 3 days plus cisplatin 45 mg/m^2 per day on days 2 and 3. In that study, 13 patients had well-differentiated carcinoid tumors, 14 had well-differentiated islet-cell carcinomas and 18 had anaplastic NENs. Among the 27 patients with well-differentiated carcinoid tumors or islet-cell carcinomas, only two partial objective tumor regressions were observed (7%). Responses up to 67% were reported. Cisplatin is generally associated with greater nephrotoxicity and ototoxicity. However, in this study toxicity was severe for most patients and included vomiting, leukopenia, thrombocytopenia, anemia, alopecia and neuropathy [23].

It is generally accepted that cisplatin and etoposide have reasonable response rates in patients with poorly differentiated/rapidly progressing NENs. However, the toxicity is considerable and nephrotoxicity is the dose-limiting factor.

In a study of what the authors described as 'heavily pretreated malignant endocrine tumors', ten out of 18 patients (56%) with foregut carcinoids responded radiologically and/or biochemically, with a median duration of 9 months, and seven out of 14 patients (50%) with malignant endocrine 'pancreatic tumors', responded radiologically and/or biochemically, with a median duration of 9 months. No difference in response was seen between patients with atypical and those with typical foregut carcinoids or between patients with well differentiated and those with poorly differentiated endocrine pancreatic carcinoma. Nineteen out of 36 patients (53%) had grade 1–2 nephrotoxicity and 23 (64%) had grade 3–4 neutropenia [24].

Fluoropyrimidines & oxaliplatin-based regimens

The combination of folinic acid, the fluoropyrimidine 5-FU and oxaliplatin (FOLFOX) is commonly used in GI malignancies such as colon cancer. Oxaliplatin–5-FU-based regimens have also been explored as a treatment for NENs in recent years. Oxaliplatin causes neuropathy and unique cold sensitivity but, if side effects are monitored closely, it is generally well tolerated.

With the wide availability and ease of use of the oral fluoropyrimidine capecitabine (Xeloda®, Roche, Basel, Switzerland), the Xeloda and oxaliplatin (XELOX) regimen is increasingly employed in GI malignancies. XELOX is generally comparable in activity to 5-FU and is well tolerated. Bajetta *et al.* reported using XELOX (oxaliplatin 130 mg/m^2 and capecitabine 2000 mg/m^2 per day) for high-grade NENs and it was observed that 70% of patients progressed on this regimen, while 23% patients achieved partial response [25]. The primary sites of the disease were: lung (n = 10), pancreas (n = 15), small bowel (n = 8), unknown (n = 1) and other (n = 6). The study enrolled NENs after progression following SSAs; 40 patients with advanced NENs were treated with XELOX. Of these, 13 had untreated, poorly differentiated NENs, 27 had well-differentiated NENs in progression after SSAs. In 13 patients with poorly differentiated NENs, the ORs were: three partial responses (PR; 23%), one stable disease (SD; 7%) and nine with progressive disease (PD; 70%). Eleven percent achieved biochemical responses. In 27 patients with well-differentiated NENs, the OR were: eight PRs (30%), 13 SDs (48%) and six PDs (22%) [25].

In another trial the combination of bevacizumab with capecitabine and oxaliplatin were evaluated in 40 patients with metastatic or unresectable neuroendocrine tumors. Only 31 patients were available for response assessment. The study included 20 pancreatic NENs, five tumors from small bowel primaries and five from unknown original sites. In the 37 patients in which pathology was reviewed, the Ki-67 index proliferation index was >20% in seven and 0–20% in 30 patients. PRs were observed in seven patients (23%), SD in 22 patients (71%) and PD in two patients (6%). Of those who achieved a PR, six had pancreatic NENs and one had an unknown primary with liver involvement. The 1-year PFS was 52% and median PFS was 13.7 months [26].

Another Phase II trial using FOLFOX plus bevacizumab showed encouraging activity in patients with advanced pancreatic NENs with a response rate of 23% (three out of 13 patients) [27].

Temozolomide & capecitabine

Temozolomide is an alkylating agent with activity in metastatic melanoma [28] and glioma [29]. It shares its active metabolite with dacarbazine, which has reported activity in NENs. Temozolomide can be administered orally and is better tolerated, thus possibly easier to use. Like dacarbazine, temozolomide is converted to the active alkylating agent through a spontaneous chemical conversion process. In the last few years, several small studies have reported promising responses to temozolomide combinations. These regimens included combinations with thalidomide, bevacizumab or everolimus, showing response rates of 45, 24 or 35%, respectively (Table 4). Given that capecitabine is another oral agent that is relatively well tolerated and has antitumor activity in NENs, the combination of capecitabine with temozolomide has also been explored. A retrospective study by Strosberg *et al.* demonstrated an impressive response rate of up to 70% using temozolomide and capecitabine [30].

Identifying a predictive biomarker that may predict response to anticancer therapy is useful in potentially enriching cohorts of patients who respond to a particular therapy and avoiding exposing those unlikely to respond to untoward side effects. *O*-6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme. Epigenetic silencing of MGMT by methylation of its promoter leads to inhibition of DNA repair [29]. In turn, this could result in better efficacy of therapies that aim to damage DNA, such as radiation or alkylating chemotherapy including that with temozolomide. MGMT is a prognostic and predictive marker in glioblastoma and its methylation leads to enhanced response to temozolomide [31]. MGMT deficiency, measured by immunohistochemistry, is more common in pancreatic NENs than in carcinoid tumors, as is treatment response to temozolomide-based therapy. Absence of MGMT may explain the sensitivity of some pancreatic NENs to treatment, as reported in a study that assessed 97 archival NEN specimens [32].

The study also looked at rates of treatment response and survival in a cohort of 101 consecutive NEN patients who had received treatment with a temozolomide-based regimen at one of three institutions. MGMT expression was directly correlated with treatment response in 21 patients who had available tumor tissue and response data. MGMT deficiency was observed in 19 out of 37 (51%) pancreatic NENs and zero out of 60 (0%) carcinoid tumors ($p < 0.0001$). In the clinical cohort, 18 out of 53 (34%) patients with pancreatic NENs but only one out of 44 (2%) patients with carcinoid tumors ($p < 0.001$) experienced a partial or complete response to temozolomide-based therapy. Among the 21 patients with evaluable tumor tissue who had also received treatment with temozolomide, four out of five with MGMT-deficient tumors (all pancreatic NENs) and zero out of 16 patients with tumors showing intact MGMT expression responded to treatment ($p = 0.001$) [32].

Other studies investigating combinations of cytotoxic chemotherapy with antiangiogenic agents include a Phase II study (n = 34) combining bevacizumab with temozolomide. In that study, there was an overall radiographic response rate of 15% (five out of 34). Not surprisingly, the response rate was higher in pancreatic NENs (33%; five out of 15), while it was zero out of 19 in other NENs. The median PFS was 11.0 months (14.3 months for pancreatic NENs compared with 7.3 months for carcinoid tumors). The median overall survival was 33.3 months (41.7 months for pancreatic NENs compared with 18.8 months for carcinoid tumors) [33].

The results of various small clinical studies of temozolomide are included in Table 4. Although the data are promising, due to the rare nature of these tumors, the trials were numerically small.

Dacarbazine (or dimethyl triazeno imidazole carboxamide)

Another cytotoxic agent that has been studied in NENs is dacarbazine. In the study by Sun *et al.*, patients crossed over to dacarbazine treatment after disease progression following first-line treatment (either FU/DOX or FU/STZ), and 73 patients were assigned to one of these three treatments based on their previous treatment or on abnormal baseline cardiac or renal function. The response rate of crossover dacarbazine treatment was 8.2%, with a median survival of 11.9 months [21].

Other combinations

The combination of irinotecan and cisplatin is known to be active in both small cell lung cancer and upper GI cancers. Therefore,

Table 4. Temozolomide combinations for neuroendocrine neoplasms.

Study (year)	Individuals (n)	pNEN (n)	Regimen	Response rate (pNEN, %)	Ref.
Kulke *et al.* (2006)	29	11	TMZ/thalidomide	25 (45)	[34]
Chan *et al.* (2012)	34	15	TMZ/Bev	15 (33)	[33]
Ekeblad *et al.* (2007)	36	12	TMZ	14 (8)	[40]
Kulke *et al.* (2010)†	24	24	TMZ/everolimus	(35)	[41]
Strosberg *et al.* (2011)	30	30	TMZ/capecitabine	(70)	[30]
Welin *et al.* (2011)	25	10 (pNEN)	TMZ/capecitabine (+ Bev in five patients)	33	[42]
Koumarianou *et al.* (2012)	15	7	mTMZ/Bev/LAR	64	[43]

†Abstract.
Bev: Bevacizumab; LAR: Long-acting octreotide; mTMZ: Metronomic temozolomide; pNEN: Pancreatic neuroendocrine neoplasm; TMZ: Temozolomide.

it was thought to be a good combination to move forward in a prospective trial of metastatic NEC. Kulke *et al.* assessed the efficacy of the combination of irinotecan 65 mg/m^2 and cisplatin 30 mg/m^2 administered weekly for 2 out of every 3 weeks [34]. One radiological response was observed out of four patients who had poorly differentiated disease compared with no responses in the 14 patients with well-differentiated tumors. The median overall survival was 11.4 months and, importantly, the toxicities associated with this regimen were mild (mainly myelosuppression, nausea and diarrhea). It was concluded that the combination of irinotecan and cisplatin may have activity in poorly differentiated tumors.

In the same year, Hainsworth *et al.* published a Phase II multicenter trial evaluating the efficacy of chemotherapy with paclitaxel, carboplatin and etoposide in advanced poorly differentiated NECs [35]. After four cycles of this combination treatment, patients who achieved an OR or SD went on to have 24 weeks of weekly paclitaxel as maintenance treatment. Of the 78 patients treated, 15% had a complete response and the overall response rate was 53%. Five patients remained disease free from 18 to 66 months after therapy. The median survival was 14.5 months. However, the authors concluded that the three-drug combination was moderately toxic and had no obvious efficacy advantage over the standard platinum/etoposide regimens.

There are many other permutations of the mentioned chemotherapeutic agents and others, such as gemcitabine, in combination. For all of these, responses between 20 and 30% in NENs have been reported, but all reports have been of small retrospective series [36,37].

Conclusion

The field of NENs, especially of GEP-NENs, is evolving, as evidenced by the steady updating of pathological classifications and guidelines worldwide as well as expanding new therapeutic options. More studies are needed to define subclasses within the G2 group of neuroendocrine tumors. This might be important to identify the appropriate treatment for each of these subclasses. A multidisciplinary approach to diagnosis and treatment is crucial in this entity that requires multifaceted expertise. While multimodal therapy is important, it is also important to carefully tailor the appropriate treatment for the appropriate individual patient. Somatostatin analogues remain important in the treatment of neuroendocrine tumors, but new techniques, such as PRRT, and new biologic therapies are being actively investigated and do have an evolving role in this disease. However, despite advances in molecular-targeted therapies and nuclear medicine techniques such as PRRT, both locoregional therapy and systemic treatment remain indispensable. There have only been a small number of controlled clinical trials on chemotherapy, caution should thus be exercised when applying results of these studies to practice.

A major obstacle in interpreting GEP-NEN study results is the heterogeneous mixture of tumor histologies. In addition, in NEN trials the tumors are usually from different primary sites and the studies are relatively small and uncontrolled. NENs at pancreatic primary sites are more likely to respond to systemic therapy. In low- (G1) and intermediate-grade (G2) NENs, there are promising biologic therapies including sunitinib and everolimus. More studies exploring biologic combinations and other novel agents such as PI3K inhibitors are currently underway.

In G3 NENs, Ki-67 index may correlate with response to chemotherapy [38]. Although G3 NENs respond to chemotherapy, these high-grade, poorly differentiated tumors have a less favorable prognosis. Treatment guidelines for G3 tumors are based on small studies and extrapolation from small cell lung cancer data using platinum with etoposide. Other regimens with probable activity include FOLFOX or oxaliplatin with capecitabine (XELOX or CAPOX). Combinations of temozolomide and capecitabine are active but mainly in pancreatic NENs.

While results from the therapeutic studies in the field are promising, they have to be interpreted with caution, as they are mostly single arm, thus there is potential for selection bias. In addition, the primary end point of some older streptozocin studies was response rate and, in some trials, the clinician assessed response by physical examination. This would not be considered acceptable according to modern clinical trial standards. Even radiology response can be misleading and robust end points such as survival should be considered. Adequately powered randomized prospective studies are needed to confirm the results of these studies. However, considering the uncommon incidence and heterogeneity of NENs, this will only be possible with effective collaborations.

Expert commentary & five-year view

Low- (G1) and intermediate-(G2) grade NENs are preferably not treated with cytotoxic chemotherapy unless patients develop significant radiologic or symptomatic tumor. Historically, STZ with 5-FU or DOX has been used in this setting. More recently, capecitabine/temozolomide combinations, generally in pancreatic NENs, have been reported active. FOLFOX and CAPOX also seem promising in these tumors. Stabilization of the disease may occur in approximately 30–50% of patients. No data exist to support the use of adjuvant therapy in pancreatic nonfunctioning NENs.

Regarding poorly differentiated NECs (G3), given the paucity of randomized studies of these tumors, there are no clear evidence-based guidelines. However, it is reasonable to treat patients according to guidelines established for small cell lung cancer, incorporating platinum (cisplatin or carboplatin)-based doublet treatment with etoposide. Although these tumors are initially highly chemosensitive, the natural history of this disease is such that relapses occur early, which ultimately leads to a poor prognosis. Further clinical trials in this group of patients need to be conducted to establish a worldwide standard of care and to improve the prognosis of this highly aggressive group of tumors. Biologic treatment targeting specific cellular abnormalities and pathways may be important for the future and are the subject of ongoing clinical research.

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Key issues

- Timely and accurate diagnosis and treatment of neuroendocrine neoplasms (NENs) are challenging clinical tasks.
- Considering difficulties in the diagnosis and treatment approach, a multidisciplinary strategy is of paramount importance.
- Cytotoxic chemotherapy is often utilized in GEP-NENs, but pancreatic NENs seem to be more sensitive to chemotherapy than NENs of nonpancreatic origin.
- Low- and intermediate-grade NENs are less sensitive to chemotherapy than high-grade poorly differentiated NENs, but low- and intermediate-grade NENs have a better prognosis than high-grade NENs.
- Almost all published NEN studies investigating cytotoxic therapies are nonrandomized and include relatively small numbers of patients.
- Regimens that have shown to be active include platinum combinations and, recently, temozolomide, as well as combinations with biologic agents such as bevacizumab and everolimus.
- Randomized adequately powered studies investigating cytotoxic chemotherapy are needed in NENs.

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