WOMEN IN CANCER THEMATIC REVIEW

# Systemic therapies in neuroendocrine tumors and novel approaches toward personalized medicine

**Marianne E Pavel**[1] and **Christine Sers**[2]

[1]Medical Department, Division of Hepatology and Gastroenterology including Metabolic Diseases, Campus Virchow Klinikum, Charité University Medicine, Berlin, Germany
[2]Institute of Pathology, Charité University Medicine, Berlin, Germany

Correspondence should be addressed to M E Pavel
**Email**
marianne.pavel@charite.de

## Abstract

Neuroendocrine tumors (NETs) are a group of heterogenous neoplasms. Evidence-based treatment options for antiproliferative therapy include somatostatin analogues, the mTOR inhibitor everolimus, the multiple tyrosine kinase inhibitor sunitinib and peptide receptor radionuclide therapy with 177-Lu-octreotate. In the absence of definite predictive markers, therapeutic decision making follows clinical and pathological criteria. As objective response rates with targeted drugs are rather low, and response duration is limited in most patients, numerous combination therapies targeting multiple pathways have been explored in the field. Upfront combination of drugs, however, is associated with increasing toxicity and has shown little benefit. Major advancements in the molecular understanding of NET based on genomic, epigenomic and transcriptomic analysis have been achieved with prognostic and therapeutic impact. New insight into molecular alterations has paved the way to biomarker-driven clinical trials and may facilitate treatment stratification toward personalized medicine in the near future. However, an improved understanding of the complexity of pathway interactions is required for successful treatment. A systems biology approach is one of the tools that may help to achieve this endeavor.

**Key Words**
- evidence-based medicine
- molecular genetics
- epigenetics
- predictive biomarker
- novel drugs

*Endocrine-Related Cancer* (2016) **23**, T135–T154



## Introduction

Neuroendocrine tumors (NET) represent a diverse group of rare neoplasms, sharing features of endocrine and neuronal cells. The incidence is 2.5–5/100,000 population, and is still increasing (Yao *et al.* 2008*a*). Most frequently, they occur in the gastrointestinal (GI) tract and pancreas, followed by the lungs. Due to their indolent course, they are often diagnosed at an advanced tumor stage, particularly if localized in the small intestine or pancreas (Lawrence *et al.* 2011). From a clinical perspective, NET can be divided into functional and non-functional tumors. Functional tumors account for one-third of all NET and are characterized by specific symptoms associated with an elevation of specific biomarkers (e.g. carcinoid syndrome in serotonin-secreting tumors, hypoglycemia in insulin-producing pancreatic NET). In the current WHO classification, NET are divided based on their histology including grading, i.e. proliferative activity (Ki-67 in % and/or mitotic count/10 high power fields) in NET G1 (≤2% Ki-67 or ≤2 mitoses), NET G2 (≥3–20% Ki-67 or <20 mitoses) and small-cell or large-cell neuroendocrine

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



This paper is part of a special section celebrating Women Working Within the Field of Cancer. The Guest Editors for this section were Charis Eng and Deborah Marsh.

carcinoma (NEC G3; >20% Ki-67 or >20 mitoses) (Bosman *et al.* 2010). One of the common features of NET G1/G2 is the expression of somatostatin receptors (sstr) that can be exploited for functional imaging (octreoscan or 68-Ga-DOTA-PET/CT) and somatostatin receptor-targeted therapy. In contrast, sstr expression in NEC G3 is rather low (van Essen *et al.* 2014). Several parameters have an impact on the prognosis and therapeutic management, most importantly Ki-67 and primary site, but also tumor burden, age at diagnosis and WHO performance status (Durante *et al.* 2009, Frilling *et al.* 2012, Rindi *et al.* 2012, Rinke *et al.* 2016). On the one hand, evidence-based medicine has changed the treatment landscape in the last 5–7 years. On the other hand, clinical and pathological criteria still guide therapeutic decisions in the absence of definite molecular predictors of response. Although several approved and non-approved drugs are available for systemic treatment, patients may develop resistance to therapy or show intrinsic resistance. The identification of predictors of tumor response is urgently required to improve the outcome and avoid unnecessary toxicity of ineffective therapies. This review summarizes established treatment options in advanced GI and pancreatic NET with a focus on antiproliferative therapies, recent endeavors to improve the outcome and novel approaches toward a more personalized medicine.

## Current treatment options: what is evidence based?

Therapeutic options include surgery, loco-regional and ablative therapies, somatostatin analogues, interferon alpha, novel targeted drugs, peptide receptor radionuclide therapy (PRRT) and systemic chemotherapy. All options are established in the management of NET, but their evidence level differs for intestinal (iNET) and pancreatic NET (pNET) (Fig. 1). Formerly the term 'carcinoid' was used not only for intestinal NET but also for NET of other origin, such as gastric, lung, thymic and other less frequent types of NET. Although the term 'carcinoid' was replaced by NET in the WHO classification of GI NET (Bosman *et al.* 2010), it is still preserved for lung carcinoids.

Surgery is the mainstay of treatment for functionally active NET and should even be considered in non-curative resectable metastatic disease to alleviate symptoms, particularly in metastatic pNET such as insulinoma or NET associated with the carcinoid syndrome. If the general condition of the patient is appropriate, non-functioning NET G1 and G2 should be considered for curative resection according to expert recommendations (ENETS consensus guidelines and NCCN guidelines) (Kulke *et al.* 2015*d*, Pavel *et al.* 2016). However, all studies are retrospective,

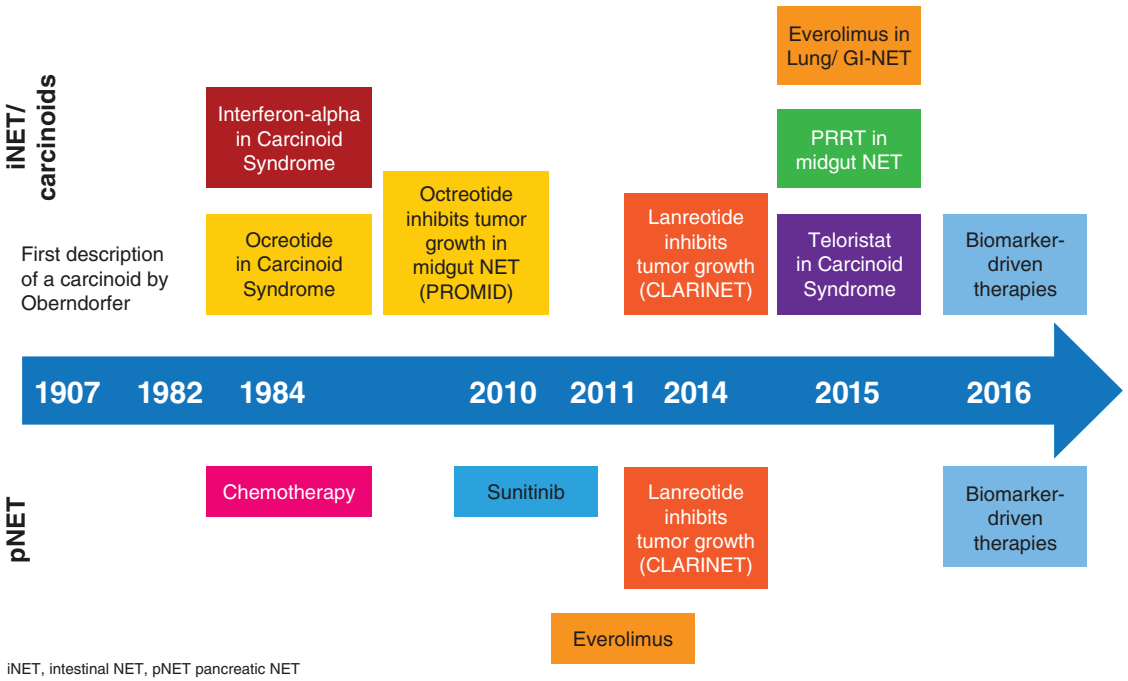


**Figure 1**
Evolution of therapies in neuroendocrine tumors.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

and up to 90% of patients have recurrent disease within 5 years if liver metastases existed before surgery (Frilling *et al.* 2012). A systematic review found no robust evidence that a liver resection was superior to any other liver-directed therapies in improving overall survival or progression-free survival (PFS) (Lesurtel *et al.* 2015). Histology, including Ki-67, tumor extent and presence of extra-abdominal disease, limits the use of surgery (Tamburrino *et al.* 2016).

Somastostatin analogues (SSA) are the mainstay of therapy for symptoms related to the carcinoid syndrome and functionally active pNET, such as VIPOMA and glucagonoma since the mid-1980s. Two commercially available SSA, octreotide and lanreotide, preferentially bind to sstr-2 and to a lesser extent to sstr-5 that are expressed in 80–90% of well-differentiated G1/G2 NET; SSA are available as long-acting release formulations; octreotide is also available as short-acting sc. formulation (Modlin *et al.* 2010). In two placebo-controlled trials (PROMID and CLARINET), the antiproliferative activity has been demonstrated by the prolongation of time to progression (TTP) and PFS, respectively for octreotide in low-grade (G1) midgut NET (TTP 14.3 months vs 6 months on placebo) and for lanreotide in entero-pancreatic NET (PFS >27 months vs 18 months on placebo) of low to intermediate grade (G1 and G2 ≤10% Ki-67) (Rinke *et al.* 2009, Caplin *et al.* 2014). Although objective remissions are rare with SSA (<2%), disease stabilization is observed in two-thirds of the patients. Lanreotide was active irrespective of Ki-67, tumor burden and primary site. The open-label extension study of the CLARINET study confirmed the antiproliferative activity in progressive disease patients on placebo who crossed over to open-label lanreotide (Caplin *et al.* 2016). Most patients had stable disease before therapy in the CLARINET study, whereas the disease status was unknown in the PROMID study. As in the CLARINET study patients on the placebo arm remained stable for a long time (median PFS in small intestinal (SI) NET 21 months, in pNET 12 months), a watch-and-wait strategy may be justified in a subgroup of patients that still needs to be defined at a molecular level. Although the somatostatin receptor as a target is broadly expressed in NET, there are no predictors of response. Patients with low Ki-67 (<5% or <10%) have probably a more durable benefit (Jann *et al.* 2013, Faggiano *et al.* 2016). Prospective data are lacking in NET G2 up to 20% Ki-67 but will be generated by an ongoing clinical trial (CLARINET forte, NCT02651987) in progressive GEP-NET. The expression of a truncated splice variant of sstr-5 was associated with poor biochemical response to octreotide in acromegaly (Marina *et al.* 2015). The finding that tumor expression of the truncated sstr-5 was associated with more aggressive behavior in pancreatic NET warrants further investigation in NET (Sampedro-Núñez *et al.* 2016). The value of SSA for NET of other origin, such as gastric or rectal NET remains unclear, their use, however, seems justified based on the expression of somatostatin receptors, and low grade or slow growth in the absence of any approved drugs. Similarly, the role of SSA in lung NET (carcinoids) is not well defined; however, clinical trials (e.g. SPINET, NCT02683941) will assess their value in the future. SSAs have a well-known and favorable long-term safety profile (Rinke *et al.* 2009, Modlin *et al.* 2010, Caplin *et al.* 2014), and this supports consideration as a first-line therapy in different types of NET.

Interferon alpha (IFN-α) has been introduced in the management of carcinoid syndrome in the mid-1980s (Öberg *et al.* 2000). Interferon-α acts by binding to specific receptors on the cell surface, and subsequently activating cytoplasmic messengers, such as Janus kinase 1 (JAK-1) and tyrosine kinase 2 (TYK-2). IFN-α inhibits the secretion of bioactive compounds including serotonin and tumor growth by multiple effects including a direct inhibitory effect on the cell cycle, inhibition of growth factors production, antiangiogenic effects and modulation of the immune response (Platanias 2005). Due to more pronounced side effects compared with SSA, it is less frequently used, unless in refractory carcinoid syndrome, and for antiproliferative purpose in selected cases, e.g. in sstr-negative NET (Pavel *et al.* 2016). In a large randomized trial with more than 400 patients with carcinoids, IFN-α of 5 million units three times per week was equally effective as bevacizumab, each in combination with octreotide (Yao *et al.* 2015).

Peptide receptor radionuclide therapy (PRRT) either with 90-yttrium-labeled compounds or more recently with 177-Lu-DOTATATE has been used for 15 years in uncontrolled trials in different types of NET demonstrating mostly stabilization of disease and in subpopulations remissions in 15–35% of patients (Brabander *et al.* 2016). Recently, the results of the first randomized controlled trial in midgut NET (NETTER-1) with 4 cycles of Lu-DOTATATE and concomitant octreotide compared with high-dose octreotide (60 mg/month) after failure of standard dose of octreotide have been reported (Strosberg *et al.* 2015). The objective response rate was 19% with PRRT and 2% with high-dose octreotide. Median PFS with PRRT was not reached (>27 months) and was 8.4 months with octreotide. Expression of sstr is a prerequisite for the use of PRRT. It remains unclear which patients have the highest benefit with respect to objective and durable



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

response; however, a strong expression of sstr-2 (Krenning Scale 3–4 as fulfilled in the NETTER-1 trial) seems to be of importance. Prospective trials to explore the role of PRRT in pNET and NET of other sites are ongoing (www.clinicaltrials.gov). Different drugs (temozolomide, capecitabine or everolimus) have been used in combination with PRRT; however, it remains unclear if combination therapies including the use of radiosensitizers will be superior to PRRT alone (Hubble *et al.* 2010, Claringbold *et al.* 2012, 2015), and further prospective clinical trials are warranted.

Among novel targeted drugs many different agents (tyrosine kinase inhibitors, anti-VEGF, IGF-R antibodies, EGFR antagonists and novel somatostatin analogues) have been explored in phase II trials (Pavel & Wiedenmann 2011); however, few drugs have advanced toward randomized controlled phase III trials, e.g. the mTOR inhibitor everolimus, and the multiple tyrosine kinase inhibitor sunitinib. Sunitinib and everolimus represent the only approved novel targeted drugs in NET. Everolimus has been most extensively studied in NET and has activity in a broad profile of patients with progressive disease. Components of the mTOR pathway are activated in NET, more frequently in pNET compared with SI NET (Kasajima *et al.* 2011). Based on prolongation of PFS, everolimus was first approved in pNET (median PFS 11 months with everolimus vs 4.6 months on placebo; RADIANT-3), and more recently in non-functional progressive intestinal and lung NET (median PFS 11 months with everolimus vs 3.9 months on placebo; RADIANT-4) (Yao *et al.* 2011*b*, 2016). In contrast, in a large trial of everolimus+octreotide in patients with carcinoid syndrome, the efficacy was less clear (Pavel *et al.* 2011). Around 60% of patients require dose reduction and 12–19% withdrew therapy in clinical trials due to side effects (Pavel *et al.* 2011, Yao *et al.* 2011*b*, 2016). Some side effects like infections may be crucial and require careful selection and surveillance of patients, thus strengthening the unmet need of predictive biomarkers.

Preclinical studies indicate that susceptibility to everolimus may vary in individual patients even if the tumor has the same site of origin (Svejda *et al.* 2011; Gagliano *et al.* 2013). As demonstrated in the RADIANT-4 study, everolimus was active in G1 and G2 NET (Yao *et al.* 2016). However, in most studies, no data of Ki-67 were available. Clinical trials exploring everolimus in G3 NEN are ongoing (EVINEC, NCT02113800, NCT02248012; www.clinicaltrials.gov). The prior use of chemotherapy had no impact on everolimus efficacy in pNET (Lombard-Bohas *et al.* 2015). The observation that 14% of patients with pNET have mutations in the mTOR pathway (Jiao *et al.* 2011) while these mutations are extremely rare in intestinal NET, however, is in contrast to the finding that a clinical benefit in many patients with a disease control rate of 73 and 81% was observed with everolimus in pNET (RADIANT-3) and GI/lung NET (RADIANT-4) and a PFS benefit of 6 and 7 months, respectively. Downregulation of mTOR pathway components (e.g. TSC-2, PTEN), a frequent finding in pNET tumor samples, might be more indicative of tumor response although correlative data to everolimus efficacy are lacking (Missiaglia *et al.* 2010). Different variables have been tested for their predictive value (the occurrence of stomatitis, an early decrease of circulating chromogranin A and the FGFR4 genotype polymorphism), but none of them represents a definite predictor of response to everolimus (Yao *et al.* 2011*a*, Serra *et al.* 2012, Cros *et al.* 2016, Rugo *et al.* 2016).

Neuroendocrine tumors are known to be highly vascular and to express vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in tumor and microenvironment. Sunitinib inhibits several tyrosine kinases, including the VEGF receptor and the PDGF receptor. Sunitinib is an approved therapy in progressive pancreatic NET based on prolongation of PFS by 6 months compared with placebo (median PFS 11.4 months with sunitinib vs 5.5 months on placebo; Raymond *et al.* 2011). Circulating cytokines (interleukin-8, soluble VEGFR-3 and stromal cell-derived factor-1α) and circulating tumor cells have been associated with outcome to sunitinib and pazopanib therapy (Ebos *et al.* 2007, Grande *et al.* 2015, Zurita *et al.* 2015), but none of these markers is validated or established in clinical practice. There is no definite evidence of efficacy of tyrosine kinase inhibitors in non-pancreatic NET yet, although phase II trials indicate some efficacy of novel tyrosine kinase inhibitors (pazopanib, axitinib) (Phan *et al.* 2015, Strosberg *et al.* 2016). Common side effects like hypertension or fatigue warrant careful selection of patients for therapy.

Systemic chemotherapy is used in pNET and NEN G3; less frequently in higher grade 2 intestinal NET (Pavel *et al.* 2016). Streptozotocin-based chemotherapy, either in combination with 5-FU or doxorubicin is an established therapy for pNET that has been approved in the US in the mid-1980s. It is especially used in G2 pNET if they are progressive or associated with higher tumor burden. Response rates of 25–42% from retrospective studies of different institutions support its activity in the era of novel targeted drugs (Dilz *et al.* 2015, Krug *et al.* 2015, Clewemar Antonodimitrakis *et al.* 2016).



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

Response to chemotherapy increases with Ki-67 index, but Ki-67 alone is an unreliable means to select patients for chemotherapy (Childs *et al.* 2016). Temozolomide-based chemotherapy, either as monotherapy or in combination with capecitabine or bevacizumab may produce response rates of 30–70% based on a limited number of mostly retrospective studies and represents an alternative approach in pNET (Koumarianou *et al.* 2015). Pending data from a prospective trial of temozolomide vs temozolomide+capecitabine in progressive pNET (NCT01824875, www.clinicaltrials.gov) will provide more evidence for its use in the near future. There is low evidence for the use of chemotherapy in non-pancreatic GI NET (carcinoids) (Lamarca *et al.* 2016). In NEC G3 cisplatin or carboplatin+etoposide is standard treatment; however, responses are short lasting (in median 4–6 months) and overall survival (OS) is poor. There is no evidence for 2nd-line therapies in NEC G3 although based on small retrospective trials from single centers, different regimens may be considered (FOLFOX; FOLFIRI, temozolomide/capecitabine) (Garcia-Carbonero *et al.* 2016).

Two novel drugs have been explored for syndrome control in patients with carcinoid syndrome refractory to standard SSA dose. Pasireotide has a broader specificity and high affinity for sstr1-3 and sstr-5 compared with octreotide or lanreotide. In a comparative phase III trial, however, pasireotide (60 mg) was not superior to octreotide LAR (40 mg) every 28 days for syndrome control at 6 months (Wolin *et al.* 2015). Telotristat ethyl, previously named telotristat etiprate (the hippurate salt of telotristat ethyl), is an oral tryptophan hydroxylase inhibitor, a rate-limiting enzyme in the synthesis of serotonin, that has demonstrated in a phase III trial (TELESTAR) a significant improvement of bowel movements compared with placebo (Kulke *et al.* 2015*a*). If approved this innovative drug will fulfill an unmet need for a subpopulation of patients suffering from tumor-related serotonin hypersecretion (Pavel *et al.* 2015, Kulke *et al.* 2015*a*).

In the absence of any comparative trials of systemic therapies, the recently updated ENETS consensus guidelines take into consideration different clinical and pathological criteria next to the evidence level of drugs to guide therapy. Nevertheless, therapy selection is frequently a very individualized approach taking into account patient-related factors including comorbidities. The presence of an increasing number of agents explored in the field and the rarity of the disease make the identification of molecular predictors of response and construction of molecularly driven trials essential.

## Limitations of clinical trials

There are different methodological or tumor-derived issues that limit the exploration of predictive biomarkers in clinical trials, among them the limitation to single biopsies and use of archival tissues, clinical trial design and morphological criteria of response assessment.

### Tissue biomarkers

Findings on archival tissues from primary tumors or metastases may be misleading as they do not necessarily reflect molecular alterations in metastases at a later time point or after a series of systemic therapies. Further, a single biopsy may miss depiction of potential heterogeneity of tumors and thus influence the validation of biomarkers owing to sampling bias (Nash *et al.* 2012). Preclinical and clinical studies indicate that tumor heterogeneity exists in NET. Interestingly, in a PDGF-deficient knockout mouse, a functionally important malignant cell heterogeneity could be revealed in pNET (Cortez *et al.* 2016). Further, heterogeneity in NET is reflected by tumors with mismatch on functional imaging using FDG-PET and somatostatin receptor imaging. The combined assessment of genomics with functional imaging may represent a novel approach toward personalized cancer therapy (Basu & Basu 2016).

### Patient selection and design

Patient homogeneity and rigorous design seem to be crucial for construction of an informative clinical trial (Halperin & Yao 2016). Some early comparative trials in the field of NET were underpowered (Faiss *et al.* 2003, Arnold *et al.* 2005). Some patients were probably included in clinical trials at very advanced disease stages; thus, the potential benefit of a drug might have been underestimated. As an example, the insulin-like growth factor-1 receptor inhibitor (MK-0646) failed to show objective responses in patients with metastatic well-differentiated NET. The poor outcome of patients with carcinoids (PFS 2.7 months, OS 10.5 months), however, indicates selection of a subpopulation with very poor prognosis (Reidy-Lagunes *et al.* 2012).

### Assessment of tumor response in clinical trials

Most prospective clinical trials use RECIST criteria to assess tumor response. However, only very few trials used the same criteria to assess the tumor growth behavior accurately before enrolment in a clinical trial,



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

e.g. the CLARINET study and a phase II trial of temsirolimus and bevacizumab (Caplin *et al.* 2014, Hobday *et al.* 2015). If the accurate tumor growth rate is unknown before a new therapy, the tumor might potentially display the same growth rate but still would be considered as being stable according to RECIST as long as 20% tumor volume increase is not exceeded, and thus, the patient will be assessed as a responder. Assessment of the tumor growth rate is a novel approach to better determine response (Ferté *et al.* 2014). Central review of radiological scans that determine the slope of progression before enrolment should be implemented in clinical trials.

## Current approaches to improve outcome

Overall, none of the available systemic treatments achieves cure, and objective response rates are low with targeted drugs (<5–10%). Prolongation of PFS is the primary endpoint in most trials. Limited response duration requires novel approaches to overcome resistance to drugs. Recent prospective randomized trials failed to demonstrate a clear survival benefit, what is in part due to the nature of the disease and high cross-over rate to open-label drug in most randomized trials (Yao *et al.* 2011*b*, Vinik *et al.* 2012, Caplin *et al.* 2014, Rinke *et al.* 2016). Different approaches to improve the outcome include combination of different drugs, the predetermined sequential use of drugs, and the use of less toxic drugs as a maintenance therapy after prior use of a more aggressive therapy. Combination of different drugs is the most frequently used approach.

### Combination therapies

From a pathophysiological point of view, it is an attractive approach to combine two or three drugs targeting different pathways in NET. Multiple combination therapies have been explored in phase I/II trials, either as a de novo combination of two targeted drugs or of targeted drugs with somatostatin analogues, and also with chemotherapy, PRRT or even loco-regional therapies of the liver. Frequently, somatostatin analogues are kept as a basic therapy beyond progression while evidence of their add-on value to 2nd- or 3rd-line therapies for antiproliferative purposes is lacking (Dasari *et al.* 2015, Kulke *et al.* 2015*b*, Yao *et al.* 2015). Some drug combinations have shown unfavorable safety profile and have not been further explored, e.g. the multiple tyrosine kinase inhibitor sorafenib and bevacizumab, or everolimus, either with the anti-IGF1R antibody cixutumumab and octreotide or with erlotinib (Bergsland *et al.* 2012, Castellano *et al.* 2013, Dasari *et al.* 2015).

Many of the phase II trials exploring combination therapies are single-arm trials, and do not provide definite evidence of a superiority of combination therapies. For example, there are no clear data of a favorable effect when combining bevacizumab with streptozotocin in pNET or with oxaliplatin in different types of NET such as carcinoids, pancreatic NET or poorly differentiated NEC (Ducreux *et al.* 2014, Kunz *et al.* 2016).

Previous randomized controlled trials that investigated SSA, either octreotide or lanreotide with IFN-α failed to demonstrate the superiority of the combination therapy compared with monotherapy, but were underpowered and enrolling heterogeneous patient populations. The use of the combination of SSA with IFN-α was associated not only with higher response rates but also with increasing toxicity (Faiss *et al.* 2003, Arnold *et al.* 2005) (Table 1). Discontinuation of treatment as a result of side effects occurred in 20% of patients receiving the combination of octreotide and IFN-alpha and in 4% of patients receiving octreotide monotherapy (Arnold *et al.* 2005).

The few drug combinations that were investigated in comparative well-designed randomized controlled trials in progressive pNET failed to demonstrate that the combination of drugs was clearly superior to monotherapy. In one of these trials, everolimus was explored in combination with a novel somatostatin analogue, pasireotide that binds to four of five somatostatin receptors and downregulates IGF-1, an upstream activator of the mTOR pathway (Kulke *et al.* 2015*c*). Another trial investigated the combination of everolimus and an angiogenesis inhibitor, bevacizumab, which binds circulating VEGF (Kulke *et al.* 2015*b*). Further the dual PI3 kinase mTOR inhibitor BEZ235 was studied to inhibit potential upstream activation of the mTOR pathway along with mTOR inhibition (Libutti *et al.* 2015) (Table 1). Although the improvement in PFS seen with the combination of everolimus and bevacizumab vs everolimus alone met the predefined statistical criteria ($P<0.15$), the PFS benefit was modest (2.7 months) and was achieved at the expense of more side effects; the other two studies failed to show any benefit in PFS. However, it should be emphasized that subpopulations of patients achieved objective response of 20 and 31% in the trials of everolimus with pasireotide or with bevacizumab, respectively. Under the condition that higher objective response rates translate into more durable response, these combinations might still be considered in individual



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

**Table 1** Combination therapies in NET in randomized controlled trials.

| Study | Drugs | Tumor type | Patients | Objective response (%) | Median PFS[d] (months) | Overall survival (months) |
|---|---|---|---|---|---|---|
| Faiss *et al.* (2003) | LAN | GEP-NET | 80 | 4 | NS (TTP) | NA |
| | IFN | | | 3.7 | | |
| | LAN + IFN | | | 7.1 | | |
| Arnold *et al.* (2005) | OCT | GEP -NET | 109 | 2 | NS | 35 |
| | OCT + IFN | | | 9.3 | | 51 (NS) |
| Yao *et al.* (2015) | OCT + BEVA | Carcinoids | 402 | 12 | 16.6 | NA |
| NCT00569127 | OCT + IFN | | | 4 | 15.4 | |
| Libutti *et al.* (2015) | EVE | pNET | 62[a] | 9.7 | 72% | NA |
| NCT01628913 | BEZ235 | | | 9.7 | 70.5% (6 months PFS rate) | |
| Kulke *et al.* (2015*c*) | EVE | pNET | 160 | 6.2 | 16.8 | >34[b] |
| COOPERATE-2 | EVE + PAS | | | 20.3 | 16.4 | >34[b] |
| NCT01374451 | | | | | | |
| Kulke *et al.* (2015*b*) | EVE + OCT | pNET | 150 | 12 | 14.0[c] | 35 |
| CALGB 80701 | EVE + OCT + BEVA | | | 31 | 16.7 | 36.7 |
| NCT01229943 | | | | | | |

[a]140 patients planned; [b]not reached; [c]the potential superiority of EB vs E was assessed using a stratified log-rank test with 90% power (1-sided 0.15) to detect a HR of 0.64; [d]if not otherwise indicated.
BEVA, bevacizumab; EVE, everolimus; GEP, gastroenteropancreatic; IFN, interferon-alpha; LAN, lanreotide; NA, not available; NET, neuroendocrine tumor; NS, statistically not significant; OCT, octreotide; PAS, pasireotide; PFS, progression free survival; pNET, pancreatic NET; TTP, time to progression.



patients if an analysis of molecular features would allow an identification of responders in terms of objective response and PFS. In contrast, BEZ235 was poorly tolerated. Treatment was discontinued in 38% of the patients due to side effects compared with 16% with everolimus alone; and the drug will not be further explored. However, novel PI3kinase-AKT-mTOR inhibitors (e.g. PKI-587, a potent novel dual inhibitor of PI3K and mTORC1/C2; inhibitors of mTORC1 and mTORC2, e.g. AZD2014, OSI-027) warrant further investigation based on preclinical studies (Freitag *et al.* 2016, Massacesi *et al.* 2016, Vandamme *et al.* 2016). Another promising combination therapy is metformin and everolimus. Metformin activates adenosine monophosphate-activated protein kinase (AMPK) that is linked with PI3K/PTEN/AKT pathway and MAPK/ERK pathway and might enhance the antiproliferative efficacy of everolimus. Both drugs are currently investigated in pNET along with octreotide (Pusceddu *et al.* 2014)

### Sequential therapies

Although SSA are widely accepted as first-line therapy in SI NET and a subset of pNET, second-line therapy in SI NET might be PRRT or everolimus based on recent results from randomized trials (Strosberg *et al.* 2015, Pavel *et al.* 2016, Yao *et al.* 2016). A population-based retrospective multi-center study from Italy indicated that everolimus use after PRRT and/ or cytotoxic chemotherapy might increase the overall toxicity of everolimus; a smaller retrospective study from the Netherlands, however, reported that the safety profile of everolimus is unaffected by prior PRRT (Panzuto *et al.* 2014, Kamp *et al.* 2013). Sequence of therapies in individual patients may be driven by tumor changes over time. Some tumors may dedifferentiate to high-grade neoplasms requiring cytotoxic chemotherapy (Paul *et al.* 2016); in contrast, in tumors responding to therapies (either chemotherapy or targeted drugs) the grade/ proliferative activity may decrease (Yao *et al.* 2008*b*) or growth factors may be downregulated (von Marschall *et al.* 2003), and consecutively, less-toxic therapies might be considered. The SEQTOR trial is a prospective trial investigating the optimal sequence of two therapies in advanced or progressive pancreatic NET and aims to clarify if either starting with everolimus or STZ/5-FU followed by cross-over upon progression is superior with respect to DCR and OS (NCT02246127, www.clinicaltrials.gov). Biomarkers will be explored in a subgroup of patients in this trial to identify predictors of response.

### Maintenance therapies

The current concept of cancer therapy follows either a continuous therapy as with molecular targeted drugs (e.g. everolimus or sunitinib) or a defined treatment period of 3–12 months with cytotoxic chemotherapy. To prolong the time to progression after termination of cytotoxic therapies or after withdrawal of targeted drugs to limit toxicity, less toxic drugs could be considered. The ongoing placebo-controlled REMINET trial (NCT02288377, www.clinicaltrials.gov) investigates

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

the role of lanreotide after up to 6 months of prior chemotherapy or targeted agents in pNET.

## Novel and future therapeutic approaches

The molecular basis of NET is still poorly understood. With exome and whole genome sequencing and integrated genomic analysis, there is a remarkable increase of knowledge about genetic/epigenetic alterations in intestinal, pancreatic and lung NET. This information may not only pave the way to an integration of molecular data into the classification of NET, that is currently simply based on grading/proliferative activity but also will allow more accurate treatment stratification or even lead to discovery of novel targets.

## Mutations in NET of different sites: current knowledge

### Pancreatic neuroendocrine tumors (pNET)

Previous understanding of pNET genetics was derived from inherited disorders induced by tumor suppressor dysfunction associated with pNET. The vast majority of pNETs are sporadic, and genes involved in the hereditary disorders have also been attributed to sporadic pNET (Crona & Skogseid 2016). Two studies using next-generation sequencing (NGS) revealed alterations; among those, *MEN1* and *ATRX/DAXX* alterations were the most frequent ones (Table 2; Jiao *et al*. 2011, Yuan *et al*. 2014). The two studies, differing in technology, study population and pNET subtype present varying frequencies for *PTEN, TSC2, TP53* and *KRAS* alterations. A direct comparison of clinicopathological features of pNET from Chinese and US patients revealed higher grade, more advanced stage and larger primary tumor size in Chinese patients, but applicability of WHO and ENETS criteria for both patient groups (Tang *et al*. 2016). Two additional studies (Kimura *et al*. 2016, Vijayvergia *et al*. 2016) confirmed mutations in *KRAS* and *TP53*. PHLDA3, a pleckstrin homology-like protein, which is a potent inhibitor of *AKT* activation (Kawase *et al*. 2009), is inactivated in 72% of pNET patients due to *PHLDA3* gene loss of heterozygosity (Ohki *et al*. 2014).

### Small intestinal neuroendocrine tumors (SI NET)

The first genomic analysis of 48 primary SI NET revealed a low mutational burden of 0.1 somatic single nucleotide variants (SSNVs) per 105 nucleotides and a very heterogeneous picture with most alterations found only in individual patients (Banck *et al*. 2013). A parallel study sequencing 55 SI NET again detected only rare recurrent genetic alterations and overlapping genes (e.g. SRC) between both studies were often detected in single patients (Table 2, Francis *et al*. 2013). A detailed investigation revealed recurrent alterations on chromosome 18 affecting *CDKN1* encoding a cyclin-dependent kinase inhibitor regulating cell cycle progression at G1 and confirming previous studies (Kytola *et al*. 2001, Lollgen *et al*. 2001, Kulke *et al*. 2008).

### Colorectal neuroendocrine tumors

A comparative investigation focusing on colorectal NEC G3 and colorectal adenocarcinomas unraveled an exceptionally high proportion of *BRAFV600E* hotspot mutations (59%) in NEC G3 including NEC with a signet ring adenocarcinoma component or NEC with conventional adenocarcinoma component (Olevian *et al*. 2016). *KRAS* mutations were only detected in 17% as compared to 43% in the adenocarcinomas. In contrast, another study in high-grade NEC reports BRAF mutations in only 9% (Klempner *et al*. 2016). Thus, colorectal neuroendocrine carcinomas comprise a distinct subgroup within the GI NET, characterized by *BRAF* and *KRAS* mutations, yet in different frequencies compared with colorectal adenocarcinoma.

**Impact on prognosis** Mutations of *ATRX/DAXX* have been associated with prognosis in pNET; however, conflicting data have been reported on this issue. Marinoni and coworkers identified loss of *ATRX/DAXX* in well-differentiated primary pNET as a negative prognostic marker associated with advanced tumor stage, metastases and reduced survival (Marinoni *et al*. 2014). In line with this, Singhi and coworkers (Singhi *et al*. 2016) identified *ATRX/DAXX* loss as a negative, independent prognostic factor for disease-free survival in more than 300 pNET patients. In contrast, Jiao and coworkers reported an improved survival for patients harboring *ATRX/DAXX* alterations also in combination with *MEN1* mutations (Jiao *et al*. 2011). These contradictory observations most likely stem not only from different sizes of the patient cohorts but also from differently classified pNET. Tang and coworkers used an extended histopathological classification algorithm to distinguish well-differentiated pNET from poorly differentiated NEC of the pancreas (Tang *et al*. 2016). In this analysis, loss of *ATRX/DAXX* was confined to well-differentiated NET, whereas loss of *TP53*, *SMAD4* and *RB* expression characterized poorly



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

**Table 2** Genetic alterations in neuroendocrine tumors.

| Study | Genes (%) | Type of alteration | No. of patients analyzed |
|---|---|---|---|
| **Pancreatic neuroendocrine tumor** | | | |
| Jiao *et al*. (2011) | *ATRX* (17.6) | NS; MS; indel | 10 (WES) |
| | *DAXX* (25) | NS; MS; indel | 58 (targeted validation) |
| | *MEN1* (44.1) | NS; MS; indel; splice site | |
| | *PIK3CA* (1.4) | MS (E545K) | |
| | *PTEN* (7.3) | MS; indel | |
| | *TP53* (4.4) | MS; indel | |
| | *TSC2* (8.8) | NS; MS; indel | |
| | + 145 genes (>1%) | NS, MS, indel | |
| Yuan *et al.* (2014) | *ATRX* (35.1) | NS; MS; indel | 27 (targeted sequencing) |
| | *DAXX* (29.7) | NS; MS; indel | |
| | *KRAS* (10.8) | MS | |
| | *MEN1* (35.1) | NS; MS | |
| | *PTEN* (18.9) | MS; indel | |
| | *TP53* (13.5) | MS; indel | |
| | *TSC2* (43.2) | MS; indel | |
| | *VHL* (40.5) | NS; MS; indel | |
| | *SMAD4* (2.7) | MS | |
| Kimura *et al*. (2016) | *KRAS* | MS | 1 (individual Sanger seq) |
| | *TP53* | | |
| | *SMAD4* | | Immunostaining for TP53 and SMAD4 |
| Ohki *et al*. (2014) | *PHLDA3* (72) | LOH | 54 (50 informative)<br>Microsatellite analysis |
| Vijayvergia *et al*. (2016) | *KRAS* (18) | MS | 11 NET (targeted sequencing) |
| | *TP53* (18) | MS | |
| | *RB* (9) | MS | |
| | *IDH1/2* (9) | MS | |
| | *ATM* (9) | Ms | |
| **Small intestinal neuroendocrine tumor** | | | |
| Banck *et al*. (2013) | *SMAD4* (43) | CNV loss | 48 (WES, array CGH) |
| | *AURKA* (9) | CNV gain | |
| | *MAP2K4* (18.7) | CNV gain/loss | |
| | *AKT1* (16.6) | CNV gain/loss | |
| | *AKT2* (12.5) | CNV gain/loss | |
| | *PDGFR* (16.6) | CNV gain | |
| | *SRC* (23) | CNV gain | |
| | *BRAF* (6.3) | CNV gain; splice site | |
| | (many more single alterations) | | |
| Francis *et al*. (2013) | *CDKN1B* (10) | Indel | 50 (WES; WGS)<br>88 for validation |
| | (many more single alterations) | | |
| Kim *et al*. (2016) | *BRAF* (14.2) | MS | 14 (IonAmpliseq Cancer Hotspot Panel NGS) |
| | *SMARCB1* (7.1) | MS | |
| | *RET* (7.1) | MS | |
| | *TP53* (14.2) | MS | |
| | *STK11* (7.1) | MS | |
| | *CCNE1* (7.1) | gain | |
| **Colorectal neuroendocrine tumor** | | | |
| Olevian *et al*. (2016) | *BRAF* (59) | MS | 32 (Sanger Seq); pure NEC, MANEC and NEC with signet ring cell adenocarcinoma |
| | *KRAS* (17) | MS | |
| Klempner *et al*. (2016) | *BRAF* (9) | MS | 109 (WES) |
| | *KRAS* (32) | MS | |
| | *NRAS* (1.8) | MS | |
| | PIK3CA | MS | |

CGH, comparative genomic hybridisation; CNV, copy number variation; indel, small insertion and/or deletion resulting in frame-shift; LOH, loss of heterozygosity; MANEC, mixed adeno-neuroendocrine carcinoma; MS, missense mutation; NS, nonsense mutation; splice site, mutation within a splice site with unknown consequence; WES, whole exome sequencing; WGS, whole genome sequencing.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

differentiated NEC, both entities clearly differing in disease-specific survival.

**Impact of genomic profiling on therapy**

Molecular profiling of NET is currently widely used within clinical trials or as an individualized approach in patients refractory to established therapies. Although the most frequently observed mutations do not present direct drug targets (e.g. *MEN1*, *ATRX/DAXX*), *ATRX/DAXX* loss results in the activation of the alternative lengthening of telomeres (ALT) pathway, a mechanism of telomere maintenance, which may offer new treatment options in the future (Schmitt *et al.* 2016). Even in case potentially druggable mutations have been identified, it remains unclear if they are of functional relevance for tumor growth.

Few studies are reported so far on tumor response in individual patients based on molecular profiling of tumor tissue. In colorectal neuroendocrine tumors, *BRAF* mutations are not routinely tested. Inhibition of BRAF has shown considerable success in melanoma, and combinatorial treatments using BRAF and MEK inhibitors were able to prolong response before resistance in melanomas (Ascierto *et al.* 2016). In two patients with high-grade rectal NEC harboring *BRAFV600E* mutations, treatment with a BRAF-MEK inhibitor combination resulted in a sustained response (Klempner *et al.* 2016) suggesting that BRAF-MEK inhibitors might be an effective therapy for a subset of patients with colorectal NEC. Whole exome sequencing of tumor tissue from 12 GEP-NET patients treated with pazopanib within a clinical trial showed that in 8 of 12 patients, mutations of cancer-related genes, including but not restricted to *TP53, CNBD1, RB1, APC, BRAF, EGFR, KRAS, SMARCB1* and *VHL* were present. Three patients with *TP53* mutations had a durable response to pazopanib, whereas one patient with a small intestinal NET harboring a *BRAF V600E* mutation did not respond to pazopanib (Park *et al.* 2016). The study is limited by the small number of patients including only 3 of 12 patients with progressive disease as best response. Molecular alterations of TP53 have been reported in 4–18% of NET and seem to be associated with more aggressive subtypes of NET. Interestingly, *TP53* mutations upregulate VEGF-A and VEGFR2. In a prospective trial in 188 evaluable patients (56% harbored *TP53* mutations) with advanced or progressive solid cancers *TP53* mutations predicted response to VEGF/VEGFR inhibitors (Wheler *et al.* 2016). These findings may guide future studies with angiogenesis inhibitors in NET.

*KRAS* mutations are a hallmark alteration of pancreatic adenocarcinoma. Three publications reported low-frequency *KRAS* mutations (10–20%) also in pNET (Yuan *et al.* 2014, Vijayvergia *et al.* 2016, Kimura *et al.* 2016). Although targeting RAS directly is a mission impossible (Cox *et al.* 2014), new options arouse from targeting RAS downstream effectors and cell cycle checkpoint control. In *NRAS* mutant melanomas (Ascierto *et al.* 2016), inhibition of MEK produced a partial response in a subset of patients. Further, inhibition of MEK and CDK4/6 was effective in a preclinical study using patient colorectal xenografts (Lee *et al.* 2016) and thus represents another option for a subset of NET patients harboring *KRAS* or *NRAS* mutations.

Previous small studies with imatinib in NET lacked objective responses or were associated with toxicity (Gross *et al.* 2006, Kindmark *et al.* 2010). In a study with 27 patients, one patient with partial response to imatinib was reported (Yao *et al.* 2007). However, in a patient with an activating mutation within the *cKIT* gene indeed a sustained response to imatinib was achieved (Perkins *et al.* 2014), thus imatinib may be considered an option in highly selected patients with *cKIT* mutation.

Mutations in the mTOR pathway occur in up to 14% of pNET. In an *in vivo* NET model and in patients treated with everolimus and octreotide, an increased AKT activation was associated with rapamycin sensitivity (Meric-Bernstam *et al.* 2012). In an exploratory biomarker analysis in more than 500 patients with human EGFR 2-positive advanced breast cancer, it could be demonstrated that patients with cancers showing *PIK3CA* mutations, PTEN loss or hyperactive PI3K pathway derive PFS benefit from everolimus (André *et al.* 2016). Biomarker analysis in subsets of patients from previous everolimus trials and from ongoing trials with mTOR pathway inhibitors will provide more insight into their predictive value in the future. Interestingly, in patients with recurrent glioma strong mutation-inducing capacity of temozolomide was reported. Exome sequencing of recurrent tumors revealed alterations in both the mTOR (*MTOR, AKT, PTEN* a.o.) and RB (*RB, CDK4,6, CDKN2A*) pathway (Johnson *et al.* 2014). Similar events might be ongoing in NET, with potential impact on sequencing of therapies and support re-biopsy as a valuable tool for directing treatment.

### Biomarker-driven therapies

The lack of real-time biopsies is a major limitation to exploit and validate potential biomarkers for therapy selection. In an ongoing phase 2 trial in GEP-NET, patients will be assigned to a targeted therapy based on a mutation profile determined in tumor tissue



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

either retrieved at surgery or from a biopsy. Patients will be treated with sunitinib (for mutations in *MEN1/PDGFR/cKIT/FLT3*) or everolimus (for mutations in *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53*) (Neychev *et al.* 2015). This mutation-targeted therapy will elucidate if a more specific therapy stratification based on biomarkers will be associated with a more durable response.

## Epigenetics in NET

Due to the lack of a 'genetic driver-signature' in most NETs, epigenetic alterations became a research focus and provided potentially prognostic and predictive biomarkers. Epigenetic modifications include DNA methylation, histone modifications and miRNAs. Recently, NET epigenome profiling has led to the identification of molecularly distinct tumor subsets in pNET and SI NET (reviewed in Cives *et al.* 2016, Stålberg *et al.* 2016).

The most comprehensive analysis integrating transcriptome (mRNA and miRNA), metabolome and selected mutations in pNET was provided by Sadanandam and coworkers (Sadanandam *et al.* 2015). A miRNA and mRNA expression analysis was performed on 51 human pNET samples and in a mouse model of pNET (RipTag2). Three subgroups of patients were identified with distinct metastatic behavior and metabolic features. The islet/insulinoma tumor (IT) subtype comprises tumors with low grade and low metastatic potential. The metastasis-like primary (MLP) subtype was enriched for genes associated with fibroblasts/stroma, stem cells and hypoxia and found in many metastatic samples. The intermediate subtype (the only one not found in the RipTag2 samples) was composed of non-functional pNET, a subgroup of which 16% was associated with distant metastases. This type harbored by far the majority of *MEN1* and *ATRX/DAXX* mutations.

Similarly, in SI NETs that are characterized by significant epigenetic dysregulation in 70–80% of tumors, three subgroups of intestinal NET with different outcomes and PFS rates were identified based on an integrated genomic, epigenomic and transcriptomic analysis (Karpathakis *et al.* 2016, Stålberg *et al.* 2016). Features of the group with the best prognosis included one of the most prevalent genomic alterations in intestinal NET, the LOH of chromosome 18 and *CDKN1B* mutations; a second group with intermediate prognosis was associated with the presence of frequent promoter methylation of tumor suppressor genes (so called CpG island methylator phenotype) and the absence of copy number variations; the third group with the worst prognosis showed multiple copy number variations. Although this molecular division of pNET and SI NET reflects well the heterogeneity of NETs observed in clinical practice, like with most other gene expression profiles, independent validation of the signatures is necessary to determine its impact on patient prognosis and potential use for therapy stratification.

Although epigenetic clustering has prognostic value in pNET and SI NET, it needs to be further explored how far epigenomic profiling may provide reliable predictive biomarkers. The enzyme O6-methylguanine DNA methyltransferase (MGMT) is a DNA repair enzyme removing alkyl-guanine adducts after chemotherapy with alkylating agents, such as streptozotocin or temozolomide. The reported frequencies of *MGMT* promoter methylation (PCR or pyrosequencing) range between 0 and 40%, and for MGMT expression, between 24 and 51% depending on the method and study (Liu *et al.* 2016). Promoter hypermethylation seems to be associated with response to temozolomide in some studies; however, prospective validation is lacking (Schmitt *et al.* 2014, Walter *et al.* 2015). Two ongoing prospective trials, one in GEP-NET (temozolomide+lanreotide, NCT02231762) and one in pNET (temozolomide vs temozolomide+capecitabine, NCT01824875) included MGMT expression and promoter methylation as a biomarker.

Epigenetic modifications can be reversed and thus represent a potential target for novel drug therapy such as inhibition of enzymes involved in DNA methylation and histone modification. Although epigenetic drugs (histone deacetylase (HDAC) inhibitors e.g. valproic acid, panobinostat and DNA methyltransferase inhibitors azacytidine, decitabine) generated promising results in NET cell lines (Alexander *et al.* 2010, Arvidsson *et al.* 2016), the few studies with HDAC inhibitors in NET patients performed so far lacked major responses. In a phase II study in 15 patients with GEP-NETs, no objective remissions were observed, but stable disease was achieved as best response with panobinostat in all patients (Jin *et al.* 2016). The DNA methylation and deacetylation inhibitor, RRx-001, is the only epigenetic drug under clinical investigation to sensitize high-grade neuroendocrine tumor patients who previously responded and now have failed to a platinum-based chemotherapy (NCT02489903, www.clinicaltrials.gov).

## Transcriptome analyses

A more pathway-oriented approach in the understanding of these complex and heterogeneous tumors became



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

apparent through transcriptomic approaches, and first data indicate that molecular profiling may guide treatment decisions (Cutler *et al.* 2016). Studies from Missiaglia and coworkers (Missiaglia *et al.* 2010) reported reduced expression of TSC2 and PTEN in a significant number of pNET samples (60%) and an association of low TSC2/PTEN levels with lower survival and shorter TTP. These results, however, were challenged by other studies that failed to demonstrate a prognostic impact of TSC2 or PTEN (Qian *et al.* 2013, Haugvik *et al.* 2016). In contrast, high expression of mTOR or its activated downstream targets was associated with worse prognosis (Qian *et al.* 2013). Craven and coworkers (Craven *et al.* 2016) identified pancreas-specific angiogenesis transcriptional signatures. *FGFR* and *VEGFR1-3* gene were upregulated in pNET and PDAC (pancreatic adenocarcinomas). On the contrary, *FGF9* (fibroblast growth factor 9), *ANGPTL3* (angiopoietin-like 3), *SCG2* (secretogranin 2) among others were specifically high in pNET. Angiopoietin-like 3 and secretogranin 2 are involved in endothelial cell migration, vessel formation and survival; FGF9, a ligand of the FGFR, correlated with EGFR inhibitor resistance in colorectal cancer (Mizukami *et al.* 2016). This indicates a clear rationale for the antiangiogenic therapy in pNET, which is based on gene expression rather than gene mutation. Targeting multiple angiogenic pathways that take into account mechanisms of escape and resistance seems to be a more promising approach (Fischer *et al.* 2007, Yao & Phan 2011, Sennino *et al.* 2012). In this respect, several TKIs targeting VEGFRs and FGFRs are currently under clinical investigation (Table 3). The complex pattern of pNET and PDAC-specific angiogenic markers suggests different mechanisms of response and resistance, which need to be underpinned by more detailed biomarker analyses (Jayson *et al.* 2016), which may have an impact on the choice of antiangiogenic drugs in the future.

**Table 3** Signaling pathways and novel drugs in clinical trials in neuroendocrine tumors.

| Pathway/targets | Drugs | Mechanism(s) | Clinical trial design | Study |
|---|---|---|---|---|
| PI3K | SAR245409 (XL-765) | PI3K/mTOR inhibitor | Phase I in solid tumors | NCT00485719 |
| | CC-223 | mTOR1 + mTOR2-inhibitors | Phase I/II in solid tumors[a], MM, NHL | NCT01177397 |
| | BYL791 + everolimus | PI3K inhibitor | Phase Ib in pNET | NCT02077933 |
| | GDC-0941 | PI3K inhibitor | Phase I in solid cancers | NCT00876109 |
| | LY2584702 + everolimus | p70S6K inhibitor | Phase I in patients with solid tumors[b] | NCT01115803 |
| RAF/MEK/ERK | Regorafenib | VEGFR1-3, c-KIT, TIE-2, PDGFR-β, FGFR-1 RET, RAF-1, BRAF and p38 MAPK inhibitor | Phase II in pNET/carcinoids | NCT02259725 |
| Retinoblastoma | LEE011 (ribociclib) | CDK inhibitors | Phase 2 in lung and GEP NET (foregut) | NCT02420691 |
| | PD0332991 (palbociclib) | | Phase 2 in pNET with overexpression of Cdk4 and/or phospho-Rb1 and/or cyclin D1 | NCT02806648 |
| VEGFR/FGFR/PDGFR | Ramucirumab + SSA | VEGFR-2 antagonist | Phase II in carcinoids | NCT02795858 |
| | Nintedanib | VEGFR1-3, FGFR 1-3 PDGFR α-/ β-inhibitor | Phase II in carcinoids/NEN | NCT02399215 |
| | Sulfatinib | VEGFR1-3, FGFR1- inhibitor | Phase III in pNET, Phase III in carcinoids | NCT02678780 |
| | Famitinib | c-Kit, VEGFR2 + 3, PDGFR, Flt1 and Flt3-inhibitor | Phase II in GEP NET | NCT01994213 |
| | Lenvatinib | VEGFR1-3, FGFR1-4, RET, c-kit, PDGFRα | Phase II in pNET/GI NET | NCT02678780 |
| | Cabozantinib | VEGFR-2, c-Met inhibitor | Phase II in pNET, carcinoids | NCT01466036 |
| | X-82 + Everolimus | VEGFR/ PDGFR inhibitor | Phase II in pNET | NCT01784861 |
| | Ziv Aflibercept | VEGF-A, VEGF-B, and PLGF trapping | Phase II in carcinoids | NCT01782443 |
| | Ziv-Aflibercept + Sapanisertib | VEGF/ PLGF trapping + mTOR1-and-2 inhibition | Phase I in solid tumors[c] | NCT02159989 |
| Ubiquitin-proteasome | SNX 5422 + Everolimus | Heat-shock protein 90 (Hsp90) inhibitor | Phase I in NET | NCT02063958 |
| | Carfilzomib | Proteasome inhibitor | Phase II in advanced NET | NCT02318784 |

[a]includes non-pancreatic NET; [b]includes advanced NET; [c]includes pancreatic NET; NHL, non Hodgkin lymphoma; MM, malignant melanoma.



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

Expression profiling also revealed novel targets such as the cyclin-dependent protein kinase CDK4 which was overexpressed in around 60% of pNET tissues. CDK4 and 6 are involved in phosphorylation of the retinoblastoma (Rb) tumor suppressor gene leading to its inactivation. Growth of a human pancreatic cell line (QGP1) was inhibited in a xenograft mouse model by a CDK4/6 inhibitor (PD0332991), which reactivates the Rb pathway (Tang *et al.* 2012). These findings paved the way for further exploration of CDK inhibitors in NET (Table 3).

Heat-shock protein 90 (Hsp90) is a molecular chaperone that has been shown to play an important role in the stabilization and activation of numerous key oncogenic client proteins. Several of the proteins that are known to be overexpressed in GEP-NET are regulated by Hsp90, including EGFR, ERBB2, IGF-1R and AKT. Preclinical data indicate that Hsp90 is a promising target for growth inhibition in NET (Gloesenkamp *et al.* 2012, Fendrich *et al.* 2014); SNX-5422, an Hsp90 inhibitor is currently investigated in a phase I trial with everolimus in advanced NET (NCT02063958, www.clinicaltrials.gov).

## Improving our understanding of the complexities of interaction of pathways

Current targeted therapies in NET include a broad spectrum of inhibitors directed against tumor-driver pathway such as PIK3CA/mTOR, MAPK and RTKs (EGFR, FGFR, PDGFR and VEGFR) (Table 3). Although in selected patients mutations may become predictive markers of response and epigenetic profiling may guide therapy, a mechanistic explanation for response or resistance is lacking for the majority of inhibitors. Furthermore, concepts such as dual inhibition of pathways to prevent signaling network-intrinsic feedback responses have been tested only to a limited extent. Chiu and coworkers (Chiu *et al.* 2010) tested a combinatorial therapy of the mTOR inhibitor rapamycin together with the EGFR inhibitor erlotinib in the RipTag2 mouse model. The strong effect that rapamycin exerted in this model was enhanced by combining rapamycin and erlotinib and lead to an increased survival of tumor-bearing mice. This analysis comprised a bona fide example of how combinatorial treatments might enhance the suppression of a pathway. However, erlotinib is an EGFR inhibitor with maximal effectiveness in *EGFR* mutant (T790M) non-small cell lung cancer (Barton *et al.* 2010), whereas in most neuroendocrine tumors, neither high level EGFR expression nor mutations have been observed (Kidd *et al.* 2013). In a preliminary analysis of a phase II study of everolimus and erlotinib in NET patients ($n=17$), no remissions were observed in the subgroup of 9 patients with carcinoids, whereas 7 patients had stable disease; pNET data were not reported, and further use was limited by toxicity (Bergsland *et al.* 2012).

Thus, signaling networks and components such as RTKs relevant in subsets of NET have to be identified to enable a rational combinatorial therapy and understand obvious resistance mechanisms. Kolch and coworkers summarized major principles of signaling dynamics, network regulation and its impact onto cancer cell proliferation and drug resistance (Kolch *et al.* 2015). A mechanistic model relevant for NET is the feedback from mTORC to the adaptor protein IRS1, intimately connected with RTKs such as EGFR and IGF1R. This feedback results in AKT activation but might also result in increased MEK-ERK activation (via IRS-stimulated RAS activation) and provide an escape mechanism during everolimus therapy. Network wiring from RTK to PIK3CA/mTOR and MEK/ERK signaling is reasonably well understood; however, the influence of genetic alterations found in neuroendocrine tumors, including *TSC2, AKT* and *PTEN* alterations onto network behavior during therapeutic interference is unknown and is currently explored in NET (http://www.sys-med.de/de/demonstratoren/maptor-net). Recently published experimental work provided novel ideas on how network behavior in the context of specific genetic alterations can have an influence on therapy (François *et al.* 2015, Schwartz *et al.* 2015, Soler *et al.* 2016, Xu *et al.* 2016). Selective inhibition of PIK3CAβ in PTEN-deficient cancers results only in a transient suppression of AKT/mTOR activity due to feedback-dependent activation of RTKs and subsequent activation of PIK3CAα (Schwartz *et al.* 2015). On the contrary, PIK3CAα inhibition in tumors harboring RTK or PI3K mutations resulted in PIK3CAβ activation. Only dual inhibition of both isoforms resulted in sustained pathway suppression and inhibition of tumor cell survival. Also, Soler and coworkers describe both a growth-suppressive and angiogenesis-suppressive effect of the selective p110 PIK3CA inhibitor (GDC-0326) in the RipTag2 mouse model for pNET upon combination with the pan-PI3K inhibitor (GDC-0941) (Soler *et al.* 2016). Although only selected papers are mentioned here, it becomes clear that for successful targeted therapy in NET, a much deeper understanding of pathway wiring, feedback control and role of the deregulated genes is urgently required. Furthermore, clinical trials with



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

both broad and selective inhibitors will provide data on distinct efficacies (Massacesi *et al.* 2016).

### Immunotherapy in neuroendocrine tumors

Immunotherapy is a rapidly evolving field in different types of cancer. Checkpoint blockade therapy targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death protein 1 pathways (PD-1/PD-L1) is being associated with dramatic tumor responses in different types of cancer including lung cancer, kidney cancer and melanoma. In advanced Merkel cell carcinoma, a rare neuroendocrine virus-linked skin cancer, pembrolizumab, an anti–PD-1 monoclonal antibody was associated with an objective response rate of 56% (Nghiem *et al.* 2016).

In NET, the anti-tumor immune response is antagonized by several tumor-related factors. Tumor-associated antigens and cytokines from NET cells, dendritic cells and tumor-associated macrophages, induce and recruit regulatory T cells, which inhibit the anti-tumor immune response (Ameri & Ferone 2012). Few patients with NET have been treated so far with checkpoint inhibitors. Best response was stable disease in a patient with carcinoid and a patient with pancreatic NET (Patnaik *et al.* 2015), and it remains unclear which NET patient subgroups might potentially benefit from this therapy. In light of the activity of pembrolizumab in small-cell lung cancer and Merkel cell carcinoma (Horn *et al.* 2016, Nghiem *et al.* 2016), the role of immunotherapy might be particularly relevant in high-grade NEC.

The Keynote-158 Study (NCT02628067, www.clinicaltrials.gov) is currently investigating pembrolizumab in multiple types of advanced solid tumors including carcinoids that have progressed on standard of care therapy, and other trials are under construction. There are limited and inconsistent data in NET on molecular markers that have been associated with response to checkpoint inhibitors such as PD-1 or PDL-1 staining in tumor tissue and stroma. In one study in neuroendocrine carcinoma (61 of pulmonary, 33 of extrapulmonary origin), PD-1/ PDL-1 staining was only found in the microenvironment, macrophages and not on tumor cells (Schultheiss *et al.* 2015). Another study in foregut and hindgut tumors reported an expression of PDL-1 in 22% of tumor tissues and was associated with worse survival, but higher response rate to chemotherapy (Kim *et al.* 2016). It is currently a matter of debate which biomarkers may best predict response to checkpoint inhibitors. Mutational burden, composition and activity of a preexisting immune infiltrate and mechanisms of tumor escape from immune surveillance are considered important components associated with the probability of response (Dijkstra *et al.* 2016).

The use of dendritic cells represents a more individualized approach of immunotherapy. Tumor vaccination has been tried in endocrine malignancies many years ago, however, with negative results (Papewalis *et al.* 2008). In the recent years, this approach has been modified and successfully explored in melanoma. It is likely that with this approach a clinical benefit will be achieved rather by combining personalized dendritic cell-based vaccination with additional strategies (Datta *et al.* 2015)

Another approach of cancer immunotherapy is the use of oncolytic viruses engineered to selectively kill tumor cells. An engineered oncolytic adenovirus (AdVince) has been developed for the treatment of liver metastases from NET. The adenovirus includes the gene promoter from human chromogranin A for selective replication in neuroendocrine cells, and a cell-penetrating peptide in the capsid for increased infectivity of tumor cells, and will be injected in the liver (Leja *et al.* 2011, Yu *et al.* 2016). A clinical trial is currently recruiting patients with NET (RADNET; NCT02749331).

## Conclusions

From small trials in heterogenous types of NET with origin in various organs the NET field moved toward well-constructed placebo-controlled trials in well-defined patient populations, mainly separated in trials for pancreatic NET and non-pancreatic (intestinal and/or lung) NET. Evidence-based trials led to approval of molecularly targeted drugs including somatostatin analogues, sunitinib and everolimus. Placebo-controlled trials provided more insight into the highly variable spontaneous tumor growth behavior of NET. The exploration of targeted drugs in a broad range of NET revealed that some NETs share commonalities with respect to drug sensitivity irrespective of their primary site. Potentially druggable mutations are low in NET and still their functional relevance needs to be validated. With increasing knowledge of the molecular background of NET and definition of distinct molecular subgroups even in patients with NET of the same site of origin, the outcome of patients might be improved by individualized stratification of patients to specific treatments in the near future. The exploration and validation of biomarkers in ongoing trials will complement all ongoing endeavors in the field of



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370

Published by Bioscientifica Ltd.

genomics and epigenomics, as well as the focus on hallmarks of cancer so far widely unexplored in NET.

**Declaration of interest**
M Pavel received honoraria for presentations and consultancy from Novartis, IPSEN, Pfizer and Lexicon. Grants from Novartis and Pfizer were provided to the institution. C Sers, nothing to declare

**Funding**
This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

## References

Alexander VM, Roy M, Steffens KA, Kunnimalaiyaan M & Chen H 2010 Azacytidine induces cell cycle arrest and suppression of neuroendocrine markers in carcinoids. *International Journal of Clinical and Experimental Medicine* **3** 95–102.

Ameri P & Ferone D 2012 Diffuse endocrine system, neuroendocrine tumors and immunity: what's new? *Neuroendocrinology* **95** 267–276. (doi:10.1159/000334612)

André F, Hurvitz S, Fasolo A, Tseng LM, Jerusalem G, Wilks S, O'Regan R, Isaacs C, Toi M, Burris H, *et al.* 2016 Molecular alterations and everolimus efficacy in human epidermal growth factor receptor 2-overexpressing metastatic breast cancers: combined exploratory biomarker analysis from BOLERO-1 and BOLERO-3. *Journal of Clinical Oncology* **34** 2115–2124. (doi:10.1200/JCO.2015.63.9161)

Arnold R, Rinke A, Klose KJ, Müller HH, Wied M, Zamzow K, Schmidt C, Schade-Brittinger C, Barth P, Moll R, *et al.* 2005 Octreotide versus octreotide plus interferon-alpha in endocrine gastroenteropancreatic tumors: a randomized trial. *Clinical Gastroenterology and Hepatology* **3** 761–771. (doi:10.1016/S1542-3565(05)00481-7)

Arvidsson Y, Johanson V, Pfragner R, Wängberg B & Nilsson O 2016 Cytotoxic effects of valproic acid on neuroendocrine tumour cells. *Neuroendocrinology* **103** 578–591. (doi:10.1159/000441849)

Ascierto PA, McArthur GA, Dréno B, Atkinson V, Liszkay G, Di Giacomo AM, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, *et al.* 2016 Cobimetinib combined with vemurafenib in advanced BRAFV600-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncology* **17** 1248–1260. (doi:10.1016/S1470-2045(16)30122-X)

Banck MS, Kanwar R, Kulkarni AA, Boora GK, Metge F, Kipp BR, Zhang L, Thorland EC, Minn KT, Tentu R, *et al.* 2013 The genomic landscape of small intestine neuroendocrine tumors. *Journal of Clinical Investigation* **123** 2502–2508. (doi:10.1172/JCI67963)

Barton S, Starling N & Swanton C 2010 Predictive molecular markers of response to epidermal growth factor receptor(EGFR) family-targeted therapies. *Current Cancer Drug Targets* **10** 799–812. (doi:10.2174/156800910793357925)

Basu B & Basu S 2016 Correlating and combining genomic and proteomic assessment with in vivo molecular functional imaging: will this be the future roadmap for personalized cancer management? *Cancer Biotherapy and Radiopharmaceuticals* **31** 75–84. (doi:10.1089/cbr.2015.1922)

Bergsland E, Watt L, Ko AH, Tempero MA, Korn WM, Kelley RK, Weber T, Siah I, Hwang J, Nakakura EK, *et al.* 2012 A phase II study to evaluate the safety and efficacy of RAD001 plus erlotinib in patients with well-differentiated neuroendocrine tumors (NET). *Journal of Clinical Oncology* **30** (Supplement 4) abstract 285.

Bosman F, Carneiro F, Hruban R & Theise N 2010 *World Health Organization (WHO) Classification of Tumours of the Digestive System.* Lyon, France: IARC Press.

Brabander T, Teunissen JJ, Van Eijck CH, Franssen GJ, Feelders RA, de Herder WW & Kwekkeboom DJ 2016 Peptide receptor radionuclide therapy of neuroendocrine tumours. *Best Practice & Research: Clinical Endocrinology & Metabolism* **30** 103–114. (doi:10.1016/j.beem.2015.10.005)

Caplin ME, Pavel M, Cwikla JB, Phan AT, Raderer M, Sedlackova E, Cadiot G, Wolin EM, Capdevila J, Wall L, *et al.* 2014 Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *New England Journal of Medicine* **371** 224–233. (doi:10.1056/NEJMoa1316158)

Caplin ME, Pavel M, Ćwikła JB, Phan AT, Raderer M, Sedláčková E, Cadiot G, Wolin EM, Capdevila J, Wall L & CLARINET Investigators 2016 Anti-tumour effects of lanreotide for pancreatic and intestinal neuroendocrine tumours: the CLARINET open-label extension study. *Endocrine-Related Cancer* **23** 191–199. (doi:10.1530/ERC-15-0490)

Castellano D, Capdevila J, Sastre J, Alonso V, Llanos M, García-Carbonero R, Manzano Mozo JL, Sevilla I, Durán I & Salazar R 2013 Sorafenib and bevacizumab combination targeted therapy in advanced neuroendocrine tumour: a phase II study of Spanish Neuroendocrine Tumour Group (GETNE0801). *European Journal of Cancer* **49** 3780–3787. (doi:10.1016/j.ejca.2013.06.042)

Childs A, Kirkwood A, Edeline J, Luong TV, Watkins J, Lamarca A, Alrifai D, Nsiah-Sarbeng P, Gillmore R, Mayer A, *et al.* 2016 Ki-67 index and response to chemotherapy in patients with neuroendocrine tumours. *Endocrine-Related Cancer* **23** 563–570. (doi:10.1530/ERC-16-0099)

Chiu CW, Nozawa H & Hanahan D 2010 Survival benefit with proapoptotic molecular and pathologic responses from dual targeting of mammalian target of rapamycin and epidermal growth factor receptor in a preclinical model of pancreatic neuroendocrine carcinogenesis. *Journal of Clinical Oncology* **28** 4425–4433. (doi:10.1200/JCO.2010.28.0198)

Cives M, Simone V, Rizzo FM & Silvestris F 2016 NETs: organ-related epigenetic derangements and potential clinical applications. *Oncotarget* **7** 57414–57429. (doi:10.18632/oncotarget.10598)

Claringbold PG & Turner JH 2015 Neuroendocrine tumor therapy with lutetium-177-octreotate and everolimus (NETTLE): a phase I study. *Cancer Biotherapy and Radiopharmaceuticals* **30** 261–269. (doi:10.1089/cbr.2015.1876)

Claringbold PG, Price RA & Turner JH 2012 Phase I-II study of radiopeptide 177Lu-octreotate in combination with capecitabine and temozolomide in advanced low-grade neuroendocrine tumors. *Cancer Biotherapy and Radiopharmaceuticals* **27** 561–569. (doi:10.1089/cbr.2012.1276)

Clewemar Antonodimitrakis P, Sundin A, Wassberg C, Granberg D, Skogseid B & Eriksson B 2016 Streptozocin and 5-fluorouracil for the treatment of pancreatic neuroendocrine tumors: efficacy, prognostic factors and toxicity. *Neuroendocrinology* **103** 345–353. (doi:10.1159/000439086)

Cortez E, Gladh H, Braun S, Bocci M, Cordero E, Björkström NK, Miyazaki H, Michael IP, Eriksson U, Folestad E, *et al.* 2016 Functional malignant cell heterogeneity in pancreatic neuroendocrine tumors revealed by targeting of PDGF-DD. *PNAS* **113** E864–E873. (doi:10.1073/pnas.1509384113A)

Cox AD, Fesik SW, Kimmelman AC, Luo J & Der CJ 2014 Drugging the undruggable RAS: mission possible? *Nature Review Drug Discovery* **13** 828–851. (doi:10.1038/nrd4389)

Craven KE, Gore J, Wilson JL & Korc M 2016 Angiogenic gene signature in human pancreatic cancer correlates with TGF-beta and inflammatory transcriptomes. *Oncotarget* **7** 323–341. (doi:10.18632/oncotarget.6345)

Crona J & Skogseid B 2016 GEP- NETS UPDATE: genetics of neuroendocrine tumors. *European Journal of Endocrinology* **174** R275–R290. (doi:10.1530/EJE-15-0972)



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



Published by Bioscientifica Ltd.

Cros J, Moati E, Raffenne J, Hentic O, Svrcek M, de Mestier L, Sbidian E, Guedj N, Bedossa P, Paradis V, *et al.* 2016 Gly388Arg FGFR4 polymorphism is not predictive of everolimus efficacy in well-differentiated digestive neuroendocrine tumors. *Neuroendocrinology* **103** 495–499. (doi:10.1159/000440724)

Cutler HS, Ogando P, Uhr JH, Gonzalez DO, Warner RR & Divino CM 2016 Use of molecular profiling to guide treatment decisions in patients with neuroendocrine tumors: preliminary results. *American Surgeon* **82** 369–375.

Dasari A, Phan A, Gupta S, Rashid A, Yeung SC, Hess K, Chen H, Tarco E, Chen H, Wei C, *et al.* 2015 Phase I study of the anti-IGF1R antibody cixutumumab with everolimus and octreotide in advanced well-differentiated neuroendocrine tumors. *Endocrine-Related Cancer* **22** 431–441. (doi:10.1530/ERC-15-0002)

Datta J, Berk E, Cintolo JA, Xu S, Roses RE & Czerniecki BJ 2015 Rationale for a multimodality strategy to enhance the efficacy of dendritic cell-based cancer immunotherapy. *Frontiers Immunology* **6** 271 (doi:10.3389/fimmu.2015.00271)

Dijkstra KK, Voabil P, Schumacher TN & Voest EE 2016 Genomics- and transcriptomics-based patient selection for cancer treatment with immune checkpoint inhibitors: a review. *JAMA Oncology* [in press]. (doi:10.1001/jamaoncol.2016.2214)

Dilz LM, Denecke T, Steffen IG, Prasad V, von Weikersthal LF, Pape UF, Wiedenmann B & Pavel M 2015 Streptozocin/5-fluorouracil chemotherapy is associated with durable response in patients with advanced pancreatic neuroendocrine tumours. *European Journal of Cancer* **51** 1253–1262. (doi:10.1016/j.ejca.2015.04.005)

Ducreux M, Dahan L, Smith D, O'Toole D, Lepère C, Dromain C, Vilgrain V, Baudin E, Lombard-Bohas C, Scoazec JY, *et al.* 2014 Bevacizumab combined with 5-FU/streptozocin in patients with progressive metastatic well-differentiated pancreatic endocrine tumours (BETTER trial) – a phase II non-randomised trial. *European Journal of Cancer* **50** 3098–3106. (doi:10.1016/j.ejca.2014.10.002)

Durante C, Boukheris H, Dromain C, Duvillard P, Leboulleux S, Elias D, de Baere T, Malka D, Lumbroso J, Guigay J, *et al.* 2009 Prognostic factors influencing survival from metastatic (stage IV) gastroenteropancreatic well-differentiated endocrine carcinoma. *Endocrine-Related Cancer* **16** 585–597. (doi:10.1677/ERC-08-0301)

Ebos JM, Lee CR, Christensen JG, Mutsaers AJ & Kerbel RS 2007 Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. *PNAS* **104** 17069–17074. (doi:10.1073/pnas.0708148104)

Faggiano A, Carratù AC, Guadagno E, Tafuto S, Tatangelo F, Riccardi F, Mocerino C, Palmieri G, Damiano V, Siciliano R, *et al.* 2016 Somatostatin analogues according to Ki67 index in neuroendocrine tumours: an observational retrospective-prospective analysis from real life. *Oncotarget* **7** 5538–4557. (doi:10.18632/oncotarget.6686)

Faiss S, Pape UF, Böhmig M, Dörffel Y, Mansmann U, Golder W, Riecken EO, Wiedenmann B & International Lanreotide and Interferon Alfa Study Group 2003 Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of metastatic neuroendocrine gastroenteropancreatic tumors – the International Lanreotide and Interferon Alfa Study Group. *Journal of Clinical Oncology* **21** 2689–2696. (doi:10.1200/JCO.2003.12.142)

Fendrich V, Wichmann S, Wiese D, Waldmann J, Lauth M, Rexin P, L-Lopez C, Schlitt HJ, Bartsch DK & Lang SA 2014 Inhibition of heat shock protein 90 with AUY922 represses tumor growth in a transgenic mouse model of islet cell neoplasms. *Neuroendocrinology* **100** 300–309. (doi:10.1159/000368610)

Ferté C, Fernandez M, Hollebecque A, Koscielny S, Levy A, Massard C, Balheda R, Bot B, Gomez-Roca C & Dromain C 2014 Tumor growth rate is an early indicator of antitumor drug activity in phase I clinical trials. *Clinical Cancer Research* **20** 246–252. (doi:10.1158/1078-0432.CCR-13-2098)

Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, Chorianopoulos E, Liesenborghs L, Koch M, De Mol M, *et al.* 2007 Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* **131** 463–475. (doi:10.1016/j.cell.2007.08.038)

Francis JM, Kiezun A, Ramos AH, Serra S, Pedamallu CS, Qian ZR, Banck MS, Kanwar R, Kulkarni AA, Karpathakis A, *et al.* 2013 Somatic mutation of CDKN1B in small intestine neuroendocrine tumors. *Nature Genetics* **45** 1483–1486. (doi:10.1038/ng.2821)

François RA, Maeng K, Nawab A, Kaye FJ, Hochwald SN & Zajac-Kaye M 2015 Targeting focal adhesion kinase and resistance to mTOR inhibition in pancreatic neuroendocrine tumors. *Journal of the National Cancer Institute* **107** djv123. (doi:10.1093/jnci/djv123)

Freitag H, Christen F, Lewens F, Grass I, Briest F, Iwaszkiewicz S, Siegmund B & Grabowski P 2016 Inhibition of mTORs catalytic site by PKI-587 is a promising therapeutic option for gastroenteropancreatic neuroendocrine tumor disease. *Neuroendocrinology* [in press]. (doi:10.1159/000448843)

Frilling A, Modlin IM, Kidd M, Russell C, Breitenstein S, Salem R, Kwekkeboom D, Lau WY, Klersy C, Vilgrain V, *et al.* Working group on neuroendocrine liver metastases 2014 recommendations for management of patients with neuroendocrine liver metastases. *Lancet Oncology* **15** e8–e21. (doi:10.1016/S1470-2045(13)70362-0)

Gagliano T, Bellio M, Gentilin E, Molè D, Tagliati F, Schiavon M, Cavallesco NG, Andriolo LG, Ambrosio MR, Rea F, *et al.* 2013 mTOR, p70S6K, AKT, and ERK1/2 levels predict sensitivity to mTOR and PI3K/mTOR inhibitors in human bronchial carcinoids. *Endocrine-Related Cancer* **20** 463–475. (doi:10.1530/ERC-13-0042)

Garcia-Carbonero R, Sorbye H, Baudin E, Raymond E, Wiedenmann B, Niederle B, Sedlackova E, Toumpanakis C, Anlauf M, Cwikla JB, *et al.* Vienna consensus conference participants 2016 ENETS consensus guidelines for high-grade gastroenteropancreatic neuroendocrine tumors and neuroendocrine carcinomas. *Neuroendocrinology* **103** 186–194 (doi:10.1159/000443172)

Gloesenkamp C, Nitzsche B, Lim AR, Normant E, Vosburgh E, Schrader M, Ocker M, Scherübl H & Höpfner M 2012 Heat shock protein 90 is a promising target for effective growth inhibition of gastrointestinal neuroendocrine tumors. *International Journal of Oncology* **40** 1659–1667. (doi:10.3892/ijo.2012.1328)

Grande E, Capdevila J, Castellano D, Teulé A, Durán I, Fuster J, Sevilla I, Escudero P, Sastre J, García-Donas J, *et al.* 2015 Pazopanib in pretreated advanced neuroendocrine tumors: a phase II, open-label trial of the Spanish task force group for neuroendocrine tumors (GETNE). *Annals of Oncology* **26** 1987–1993. (doi:10.1093/annonc/mdv252)

Gross DJ, Munter G, Bitan M, Siegal T, Gabizon A, Weitzen R, Merimsky O, Ackerstein A, Salmon A, Sella A, *et al.* Israel Glivec in Solid Tumors Study Group 2006 The role of imatinib mesylate (Glivec) for treatment of patients with malignant endocrine tumors positive for c-kit or PDGF-R. *Endocrine-Related Cancer* **13** 535–540 (doi:10.1677/erc.1.01124)

Halperin DM & Yao JC 2016 Clinical trial design in neuroendocrine tumors. *Hematology/Oncology Clinics of North America* **30** 209–217. (doi:10.1016/j.hoc.2015.09.011)

Haugvik SP, Vodák D, Haugom L, Hovig E, Gladhaug IP, Heim S & Micci F 2016 Transcriptomic profiling of tumor aggressiveness in sporadic nonfunctioning pancreatic neuroendocrine neoplasms. *Pancreas* **45** 1196–1203. (doi:10.1097/MPA.0000000000000610)

Hobday TJ, Qin R, Reidy-Lagunes D, Moore MJ, Strosberg J, Kaubisch A, Shah M, Kindler HL, Lenz HJ, Chen H, *et al.* 2015 Multicenter phase II trial of temsirolimus and bevacizumab in pancreatic neuroendocrine tumors. *Journal of Clinical Oncology* **33** 1551–1556. (doi:10.1200/JCO.2014.56.2082)

Horn L, Reck M & Spigel DR 2016 The future of immunotherapy in the treatment of small cell lung cancer. *Oncologist* **21** 910–921. (doi:10.1634/theoncologist.2015-0523)



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



Published by Bioscientifica Ltd.

Hubble D, Kong G, Michael M, Johnson V, Ramdave S & Hicks RJ 2010 177Lu-octreotate, alone or with radiosensitising chemotherapy, is safe in neuroendocrine tumour patients previously treated with high-activity 111In-octreotide. *European Journal of Nuclear Medicine and Molecular Imaging*. **37** 1869–1875. (doi:10.1007/s00259-010-1483-4)

Jann H, Denecke T, Koch M, Pape UF, Wiedenmann B & Pavel M 2013 Impact of octreotide long-acting release on tumour growth control as a first-line treatment in neuroendocrine tumours of pancreatic origin. *Neuroendocrinology* **98** 137–143. (doi:10.1159/000353785)

Jayson GC, Kerbel R, Ellis LM & Harris AL 2016 Antiangiogenic therapy in oncology: current status and future directions. *Lancet* **388** 518–529. (doi:10.1016/S0140-6736(15)01088-0)

Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA, *et al*. 2011 DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* **331** 1199–1203. (doi:10.1126/science.1200609)

Jin N, Lubner SJ, Mulkerin DL, Rajguru S, Carmichael L, Chen H, Holen KD & LoConte NK 2016 A phase II trial of a histone deacetylase inhibitor panobinostat in patients with low-grade neuroendocrine tumors. *Oncologist* **21** 785–786. (doi:10.1634/theoncologist.2016-0060)

Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, Fouse SD, Yamamoto S, Ueda H, Tatsuno K, *et al*. 2014 Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science* **343** 189–193. (doi:10.1126/science.1239947)

Kamp K, Gumz B, Feelders RA, Kwekkeboom DJ, Kaltsas G, Costa FP & de Herder WW 2013 Safety and efficacy of everolimus in gastrointestinal and pancreatic neuroendocrine tumors after (177) Lu-octreotate. *Endocrine-Related Cancer* **20** 825–831. (doi:10.1530/ERC-13-0254)

Karpathakis A, Dibra H, Pipinikas C, Feber A, Morris T, Francis J, Oukrif D, Mandair D, Pericleous M, Mohmaduvesh M, *et al*. 2016 Prognostic impact of novel molecular subtypes of small intestinal neuroendocrine tumor. *Clinical Cancer Research* **22** 250–258. (doi:10.1158/1078-0432.CCR-15-0373)

Kasajima A, Pavel M, Darb-Esfahani S, Noske A, Stenzinger A, Sasano H, Dietel M, Denkert C, Röcken C, Wiedenmann B, *et al*. 2011 mTOR expression and activity patterns in gastroenteropancreatic neuroendocrine tumours. *Endocrine-Related Cancer* **18** 181–192. (doi:10.1677/ERC-10-0126)

Kawase T, Ohki R, Shibata T, Tsutsumi S, Kamimura N, Inazawa J, Ohta T, Ichikawa H, Aburatani H, Tashiro F, *et al*. 2009 PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt. *Cell* **136** 535–550. (doi:10.1016/j.cell.2008.12.002)

Kidd M, Schimmack S, Lawrence B, Alaimo D & Modlin IM 2013 EGFR/TGFα and TGFβ/CTGF signaling in neuroendocrine neoplasia: theoretical therapeutic targets. *Neuroendocrinology* **97** 35–44. (doi:10.1159/000334891)

Kim ST, Lee SJ, Park SH, Park JO, Lim HY, Kang WK, Lee J & Park YS 2016 Genomic profiling of metastatic gastroenteropancreatic neuroendocrine tumor (GEP-NET) patients in the personalized-medicine era. *Journal of Cancer* **7** 1044–1048. (doi:10.7150/jca.14815)

Kimura T, Miyamoto H, Fukuya A, Kitamura S, Okamoto K, Kimura M, Muguruma N, Ikemoto T, Shimada M, Yoneda A, *et al*. 2016 Neuroendocrine carcinoma of the pancreas with similar genetic alterations to invasive ductal adenocarcinoma. *Clinical Journal of Gastroenterology* **9** 261–265. (doi:10.1007/s12328-016-0655-6)

Kindmark H, Janson ET, Gustavsson B, Eriksson C, Larsson G, Granberg D, Kozlowacki G, Skogseid B, Welin S, Oberg K, *et al*. 2010 Five patients with malignant endocrine tumors treated with imatinib mesylate (Glivec). *Acta Oncologica* **49** 100–101. (doi:10.3109/02841860902897707)

Klempner SJ, Gershenhorn B, Tran P, Lee TK, Erlander MG, Gowen K, Schrock AB, Morosini D, Ross JS, Miller VA, *et al*. 2016 BRAFV600E mutations in high-grade colorectal neuroendocrine tumors may predict responsiveness to BRAF-MEK combination therapy. *Cancer Discovery* **6** 594–600. (doi:10.1158/2159-8290.CD-15-1192)

Kolch W, Halasz M, Granovskaya M & Kholodenko BN 2015 The dynamic control of signal transduction networks in cancer cells. *Nature Reviews Cancer* **15** 515–527. (doi:10.1038/nrc3983)

Koumarianou A, Kaltsas G, Kulke MH, Oberg K, Strosberg JR, Spada F, Galdy S, Barberis M, Fumagalli C, Berruti A, *et al*. 2015 Temozolomide in advanced neuroendocrine neoplasms: pharmacological and clinical aspects. *Neuroendocrinology* **101** 274–288. (doi:10.1159/000430816)

Krug S, Boch M, Daniel H, Nimphius W, Müller D, Michl P, Rinke A & Gress TM 2015 Streptozocin-based chemotherapy in patients with advanced neuroendocrine neoplasms – predictive and prognostic markers for treatment stratification. *PLoS ONE* **10** e0143822. (doi:10.1371/journal.pone.0143822)

Kulke MH, Freed E, Chiang DY, Philips J, Zahrieh D, Glickman JN & Shivdasani RA 2008 High-resolution analysis of genetic alterations in small bowel carcinoid tumors reveals areas of recurrent amplification and loss. *Genes Chromosomes Cancer* **47** 591–603. (doi:510.1002/gcc.20561) (doi:10.1002/gcc.20561)

Kulke MH, Hörsch D, Caplin M, Anthony L, Bergsland E, Öberg K, Welin S, Warner R, Lombard-Bohas C, Kunz P, *et al*. 2015*a* 37LBA Telotristat etiprate is effective in treating patients with carcinoid syndrome that is inadequately controlled by somatostatin analog therapy (the phase 3 TELESTAR clinical trial). *European Journal of Cancer* **51** S728 (doi:10.1016/S0959-8049(16)31951-7)

Kulke M, Niedzwiecki D, Foster N, Fruth B, Kunz P, Kennecke H, Wolin E & Venook A 2015*b* Randomized phase II study of everolimus (E) versus everolimus plus bevacizumab (E+B) in patients (Pts) with locally advanced or metastatic pancreatic neuroendocrine tumors (pNET), CALGB 80701 (Alliance). *Journal of Clinical Oncology* **33** (Supplement) abstract 4005. (available at: http://meeting.ascopubs.org/cgi/content/abstract/33/15_suppl/4005)

Kulke M, Ruszniewski P, Van Cutsem E, Valle JW, De Herder W, Pavel M, Degtyarev E, Lam DH, Salazar R, Borbath I, *et al*. 2015*c* A randomized open-label phase II study of everolimus alone or in combination woith pasireottide LAR in advanced progressive pancreatic neuroendocrine tumors (pNET): COOPERATE-2 trial. In *12th Annual European Neuroendocrine Tumor Society Conference for the Diagnosis and Treatment of Neuroendocrine Tumor Disease*. Barcelona, Spain, March 11–13, 2015

Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, Emerson L, Engstrom PF, Fanta P, Giordano T, *et al*. 2015*d* National comprehensive cancer network. Neuroendocrine tumors, version 1.2015. *Journal of the National Comprehensive Cancer Network* **13** 78–108. (available at: http://www.jnccn.org/content/13/1/78)

Kunz PL, Balise RR, Fehrenbacher L, Pan M, Venook AP, Fisher GA, Tempero MA, Ko AH, Korn WM, Hwang J, *et al*. 2016 Oxaliplatin-fluoropyrimidine chemotherapy plus bevacizumab in advanced neuroendocrine tumors: an analysis of 2 phase II trials. *Pancreas* **45** 1394–1400. (doi:10.1097/mpa.0000000000000659)

Kytola S, Hoog A, Nord B, Cedermark B, Frisk T, Larsson C & Kjellman M 2001 Comparative genomic hybridization identifies loss of 18q22-qter as an early and specific event in tumorigenesis of midgut carcinoids. *American Journal of Pathology* **158** 1803–1808. (doi:10.1016/S0002-9440(10)64136-3)

Lamarca A, Elliott E, Barriuso J, Backen A, McNamara MG, Hubner R & Valle JW 2016 Chemotherapy for advanced non-pancreatic well-differentiated neuroendocrine tumours of the gastrointestinal tract, a systematic review and meta-analysis: a lost cause? *Cancer Treatment Reviews* **44** 26–41. (doi:10.1016/j.ctrv.2016.01.005)

Lawrence B, Gustafsson BI, Chan A, Svejda B, Kidd M & Modlin IM 2011 The epidemiology of gastroenteropancreatic neuroendocrine tumors. *Endocrinology Metabolism Clinics of North America* **40** 1–18 (doi:10.1016/j.ecl.2010.12.005)



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



Published by Bioscientifica Ltd.

Lee MS, Helms T, Feng N, Gay J, Chang QE, Tian F, Wu JY, Toniatti C, Heffernan TP, Powis G, *et al.* 2016 Efficacy of the combination of MEK and CDK4/6 inhibitors in vitro and in vivo in KRAS mutant colorectal cancer models. *Oncotarget* article 9153. (doi:10.18632/oncotarget.9153)

Leja J, Yu D, Nilsson B, Gedda L, Zieba A, Hakkarainen T, Åkerström G, Öberg K, Giandomenico V & Essand M 2011 Oncolytic adenovirus modified with somatostatin motifs for selective infection of neuroendocrine tumor cells. *Gene Therapy* **18** 1052–1062. (doi:10.1038/gt.2011.54)

Lesurtel M, Nagorney DM, Mazzaferro V, Jensen RT & Poston GJ 2015 When should a liver resection be performed in patients with liver metastases from neuroendocrine tumours? A systematic review with practice recommendations. *HPB* **17** 17–22. (doi:10.1111/hpb.12225)

Libutti SK, Garcia-Carbonero R, Wolin E, Custodio Carretero A, Yao J, Hendifar AE, Tavorath R, Mukherjee N, Herbst F, *et al.* 2015 Randomized phase II study of BEZ235 or everolimus in advanced pancreatic neuroendocrine tumors. In *12th Annual ENETS Conference*, Barcelona, Spain, March 11–13, 2015.

Liu IH, Ford JM & Kunz PL 2016 DNA-repair defects in pancreatic neuroendocrine tumors and potential clinical applications. *Cancer Treatment Reviews* **44** 1–9. (doi:10.1016/j.ctrv.2015.11.006)

Lollgen RM, Hessman O, Szabo E, Westin G & Akerstrom G 2001 Chromosome 18 deletions are common events in classical midgut carcinoid tumors. *International Journal of Cancer* **92** 812–815. (doi:10.1002/ijc.1276)

Lombard-Bohas C, Yao JC, Hobday T, Van Cutsem E, Wolin EM, Panneerselvam A, Stergiopoulos S, Shah MH, Capdevila J & Pommier R 2015 Impact of prior chemotherapy use on the efficacy of everolimus in patients with advanced pancreatic neuroendocrine tumors: a subgroup analysis of the phase III RADIANT-3 trial. *Pancreas* **44** 181–189. (doi:10.1097/MPA.0000000000000262)

Marina D, Burman P, Klose M, Casar-Borota O, Luque RM, Castaño JP & Feldt-Rasmussen U 2015 Truncated somatostatin receptor 5 may modulate therapy response to somatostatin analogues-observations in two patients with acromegaly and severe headache. *Growth Hormone & IGF Research* **25** 262–267. (doi:10.1016/j.ghir.2015.07.003)

Marinoni I, Kurrer AS, Vassella E, Dettmer M, Rudolph T, Banz V, Hunger F, Pasquinelli S, Speel EJ & Perren A 2014 Loss of DAXX and ATRX are associated with chromosome instability and reduced survival of patients with pancreatic neuroendocrine tumors. *Gastroenterology* **146** 453–460.e5. (doi:10.1053/j.gastro.2013.10.020)

Massacesi C, Di Tomaso E, Urban P, Germa C, Quadt C, Trandafir L, Aimone P, Fretault N, Dharan B, Tavorath R, *et al.* 2016 PI3K inhibitors as new cancer therapeutics: implications for clinical trial design. *OncoTargets Therapy* **9** 203–210. (doi:10.2147/OTT.S89967)

Meric-Bernstam F, Akcakanat A, Chen H, Do KA, Sangai T, Adkins F, Gonzalez-Angulo AM, Rashid A, Crosby K, Dong M, *et al.* 2012 PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. *Clinical Cancer Research* **18** 1777–1789. (doi:10.1158/1078-0432.CCR-11-2123)

Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, Piemonti L, Capurso G, Di Florio A, delle Fave G, *et al.* 2010 Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *Journal of Clinical Oncology* **28** 245–255. (doi:10.1200/JCO.2008.21.5988)

Mizukami T, Togashi Y, Naruki S, Banno E, Terashima M, de Velasco MA, Sakai K, Yoneshige A, Hayashi H, Fujita Y, *et al.* 2016 Significance of FGF9 gene in resistance to anti-EGFR therapies targeting colorectal cancer: A subset of colorectal cancer patients with FGF9 upregulation may be resistant to anti-EGFR therapies. *Molecular Carcinogenesis* [in press]. (doi:10.1002/mc.22476)

Modlin IM, Pavel M, Kidd M & Gustafsson B 2010 Review article: somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours. *Alimentary Pharmacology & Therapeutics* **31** 169–188. (doi:10.1111/j.1365-2036.2009.04174.x)

Nash I 2012 Intratumor heterogeneity and branched evolution. *New England Journal of Medicine* **366** 2132–2133. (doi:10.1056/NEJMc1204069#SA3)

Neychev V, Steinberg SM, Cottle-Delisle C, Merkel R, Nilubol N, Yao J, Meltzer P, Pacak K, Marx S & Kebebew E 2015 Mutation-targeted therapy with sunitinib or everolimus in patients with advanced low-grade or intermediate-grade neuroendocrine tumours of the gastrointestinal tract and pancreas with or without cytoreductive surgery: protocol for a phase II clinical trial. *BMJ Open* **5** e008248. (doi:10.1136/bmjopen-2015-008248)

Nghiem PT, Bhatia S, Lipson EJ, Kudchadkar RR, Miller NJ, Annamalai L, Berry S, Chartash EK, Daud A, Fling SP, *et al.* 2016 PD-1 Blockade with pembrolizumab in advanced merkel-cell carcinoma. *New England Journal of Medicine* **374** 2542–2552. (doi:10.1056/NEJMoa1603702)

Öberg K 2000 Interferon in the management of neuroendocrine GEP-tumors: a review. *Digestion* **62** 92–97. (doi:10.1159/000051862)

Ohki R, Saito K, Chen Y, Kawase T, Hiraoka N, Saigawa R, Minegishi M, Aita Y, Yanai G, Shimizu H, *et al.* 2014 PHLDA3 is a novel tumor suppressor of pancreatic neuroendocrine tumors. *PNAS* **111** E2404–E2413. (doi:10.1073/pnas.1319962111)

Olevian DC, Nikiforova MN, Chiosea S, Sun W, Bahary N, Kuan SF & Pai RK 2016 Colorectal poorly differentiated neuroendocrine carcinomas frequently exhibit BRAF mutations and are associated with poor overall survival. *Human Pathology* **49** 124–134. (doi:10.1016/j.humpath.2015.11.004)

Panzuto F, Rinzivillo M, Fazio N, de Braud F, Luppi G, Zatelli MC, Lugli F, Tomassetti P, Riccardi F, Nuzzo C, *et al.* 2014 Real-world study of everolimus in advanced progressive neuroendocrine tumors. *Oncologist* **19** 966–974. (doi:10.1634/theoncologist.2014-0037)

Papewalis C, Wuttke M, Seissler J, Meyer Y, Kessler C, Jacobs B, Ullrich E, Willenberg HS, Schinner S, Baehring T, *et al.* 2008. Dendritic cell vaccination with xenogenic polypeptide hormone induces tumor rejection in neuroendocrine cancer. *Clinical Cancer Research* **14** 4298–4305. (doi:10.1158/1078-0432.CCR-08-0587)

Park C, Ha SY, Kim ST, Kim HC, Heo JS, Park YS, Lauwers G, Lee J & Kim KM 2016 Identification of the BRAF V600E mutation in gastroenteropancreatic neuroendocrine tumors. *Oncotarget* **7** 4024–4035. (doi:10.18632/oncotarget.6602)

Patnaik A, Kang SP, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, Drengler R, Chen C, Smith L, Espino G, *et al.* 2015 Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clinical Cancer Research* **21** 4286–4293. (doi:10.1158/1078-0432)

Paul D, Ostwal V, Bose S, Basu S & Gupta S 2016 Personalized treatment approach to gastroenteropancreatic neuroendocrine tumors: a medical oncologist's perspective. *European Journal of Gastroenterology and Hepatology* **28** 985–990. (doi:10.1097/MEG.0000000000000674)

Pavel ME & Wiedenmann B 2011 Novel therapeutic agents for the treatment of gastroenteropancreatic neuroendocrine tumors. *Hormone Metabolic Research* **43** 844–853. (doi:10.1055/s-0031-1291368)

Pavel ME, Hainsworth JD, Baudin E, Peeters M, Hörsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, *et al.* 2011 Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet* **378** 2005–2012. (doi:10.1016/S0140-6736(11)61742-X)

Pavel M, Hörsch D, Caplin M, Ramage J, Seufferlein T, Valle J, Banks P, Lapuerta P, Sands A, Zambrowicz B, *et al.* 2015 Telotristat etiprate for carcinoid syndrome: a single-arm, multicenter trial. *Journal of Clinical Endocrinology and Metabolism* **100** 1511–1599. (doi:10.1210/jc.2014-2247)

Pavel M, O'Toole D, Costa F, Capdevila J, Gross D, Kianmanesh R, Krenning E, Knigge U, Salazar R, Pape U-F, *et al.* 2016 Consensus



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



Published by Bioscientifica Ltd.

guidelines update for the management of distant metastatic disease of intestinal, pancreatic, bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. *Neuroendocrinology* **103** 172–185. (doi:10.1159/000443167)

Perkins J, Boland P, Cohen SJ, Olszanski AJ, Zhou Y, Engstrom P & Astsaturov I 2014 Successful imatinib therapy for neuroendocrine carcinoma with activating Kit mutation: a case study. *Journal of the National Comprehensive Cancer Network* **12** 847–852. (available at: http://www.jnccn.org/content/12/6/847)

Phan AT, Halperin DM, Chan JA, Fogelman DR, Hess KR, Malinowski P, Regan E, Ng CS, Yao JC & Kulke MH 2015 Pazopanib and depot octreotide in advanced, well-differentiated neuroendocrine tumours: a multicentre, single-group, phase 2 study. *Lancet Oncology* **16** 695–703. (doi:10.1016/S1470-2045(15)70136-1)

Platanias LC 2005 Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature Reviews Immunology* **5** 375–386. (doi:10.1038/nri1604)

Pusceddu S, de Braud F, Concas L, Bregant C, Leuzzi L, Formisano B & Buzzoni R 2014 Rationale and protocol of the MetNET-1 trial, a prospective, single center, phase II study to evaluate the activity and safety of everolimus in combination with octreotide LAR and metformin in patients with advanced pancreatic neuroendocrine tumors. *Tumori* **100** e286–e289. (doi:10.1700/1778.19298)

Qian ZR, Ter-Minassian M, Chan JA, Imamura Y, Hooshmand SM, Kuchiba A, Morikawa T, Brais LK, Daskalova A, Heafield R, *et al.* 2013 Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. *Journal of Clinical Oncology* **31** 3418–3425. (doi:10.1200/JCO.2012.46.6946)

Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, *et al.* 2011 Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *New England Journal of Medicine* **364** 501–513. (doi:10.1056/NEJMoa1003825)

Reidy-Lagunes DL, Vakiani E, Segal MF, Hollywood EM, Tang LH, Solit DB, Pietanza MC, Capanu M & Saltz LB 2012 A phase 2 study of the insulin-like growth factor-1 receptor inhibitor MK-0646 in patients with metastatic, well-differentiated neuroendocrine tumors. *Cancer* **118** 4795–4800. (doi:10.1002/cncr.27459)

Rindi G, Falconi M, Klersy C, Albarello L, Boninsegna L, Buchler MW, Capella C, Caplin M, Couvelard A, Doglioni C, *et al.* 2012 TNM staging of neoplasms of the endocrine pancreas: results from a large international cohort study. *Journal of the National Cancer Institute* **104** 764–777. (doi:10.1093/jnci/djs208)

Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M, *et al.* 2009 Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of Clinical Oncology* **27** 4656–4663. (doi:10.1200/JCO.2009.22.8510)

Rinke A, Wittenberg M, Schade-Brittinger C, Aminossadati B, Ronicke E, Gress TM, Müller HH & Arnold R 2016 Placebo controlled, double blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors (PROMID): results on long term survival. *Neuroendocrinology* [in press]. (doi:10.1159/000443612)

Rugo HS, Hortobagyi GN, Yao J, Pavel M, Ravaud A, Franz D, Ringeisen F, Gallo J, Rouyrre N & Anak O 2016 Meta-analysis of stomatitis in clinical studies of everolimus: incidence and relationship with efficacy. *Annals of Oncology* **27** 519–525. (doi:10.1093/annonc/mdv595)

Sadanandam A, Wullschleger S, Lyssiotis CA, Grötzinger C, Barbi S, Bersani S, Körner J, Wafy I, Mafficini A, Lawlor RT, *et al.* 2015 A cross-species analysis in pancreatic neuroendocrine tumors reveals molecular subtypes with distinctive clinical, metastatic, developmental, and metabolic characteristics. *Cancer Discovery* **5** 1296–1313. (doi:10.1158/2159-8290)

Sampedro-Núñez M, Luque RM, Ramos-Levi AM, Gahete MD, Serrano-Somavilla A, Villa-Osaba A, Adrados M, Ibáñez-Costa A, Martín-Pérez E & Culler MD 2016 Presence of sst5TMD4, a truncated splice variant of the somatostatin receptor subtype 5, is associated to features of increased aggressiveness in pancreatic neuroendocrine tumors. *Oncotarget* **7** 6593–6608. (doi:10.18632/oncotarget.6565)

Schmitt AM, Pavel M, Rudolph T, Dawson H, Blank A, Komminoth P, Vassella E & Perren A 2014 Prognostic and predictive roles of MGMT protein expression and promoter methylation in sporadic pancreatic neuroendocrine neoplasms. *Neuroendocrinology* **100** 35–44. (doi:10.1159/000365514)

Schmitt AM, Marinoni I, Blank A & Perren A 2016 New genetics and genomic data on pancreatic neuroendocrine tumors: implications for diagnosis, treatment, and targeted therapies. *Endocrine Pathology* **27** 200–204. (doi:10.1007/s12022-016-9447-2)

Schultheiss AM, Scheel AH, Ozretić L, George J, Thomas RK, Hagemann T, Zander T, Wolf J & Buettner R 2015 PD-L1 expression in small cell neuroendocrine carcinomas. *European Journal of Cancer* **51** 421–426. (doi:10.1016/j.ejca.2014.12.006)

Schwartz S, Wongvipat J, Trigwell CB, Hancox U, Carver BS, Rodrik-Outmezguine V, Will M, Yellen P, de Stanchina E, *et al.* 2015 Feedback suppression of PI3Kα signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kβ. *Cancer Cell* **27** 109–122. (doi:10.1016/j.ccell.2014.11.008)

Sennino B, Ishiguro-Oonuma T, Wei Y, Naylor RM, Williamson CW, Bhagwandin V, Tabruyn SP, You WK, Chapman HA, Christensen JG, *et al.* 2012 Suppression of tumor invasion and metastasis by concurrent inhibition of c-Met and VEGF signaling in pancreatic neuroendocrine tumors. *Cancer Discovery* **2** 270–287. (doi:10.1158/2159-8290.CD-11-0240)

Serra S, Zheng L, Hassan M, Phan AT, Woodhouse LJ, Yao JC, Ezzat S & Asa SL 2012 The FGFR4-G388R single-nucleotide polymorphism alters pancreatic neuroendocrine tumor progression and response to mTOR inhibition therapy. *Cancer Research* **72** 5683–5691. (doi:10.1158/0008-5472.CAN-12-2102)

Singhi AD, Liu TC, Roncaioli JL, Cao D, Zeh HJ, Zureikat AH, Tsung A, Marsh JW, Lee KK, Hogg ME, *et al.* 2016 Alterative lengthening of telomeres and loss of DAXX/ATRX expression predicts metastatic disease and poor survival in patients with pancreatic neuroendocrine tumors. *Clinical Cancer Research* [in press]. (doi:10.1158/1078-0432.ccr-16-1113)

Soler A, Figueiredo AM, Castel P, Martin L, Monelli E, Angulo-Urarte A, Mila-Guasch M, Viñals F, Baselga J, Casanovas O, *et al.* 2016 Therapeutic benefit of selective inhibition of p110α PI3-kinase in pancreatic neuroendocrine tumors. *Clinical Cancer Research* [in press]. (doi:10.1158/1078-0432.ccr-15-3051)

Stålberg P, Westin G & Thirlwell C 2016 Genetics and epigenetics in small intestinal neuroendocrine tumours. *Journal of Internal Medicine* [in press]. (doi:10.1111/joim.12526)

Strosberg J, Wolin E, Chasen B, Kulke M, Bushnell D, Caplin M, Baum RP, Mittra E, Hobday T, Hendifar A, *et al.* 2015 6LBA 177-Lu-Dotatate significantly improves progression-free survival in patients with midgut neuroendocrine tumours: results of the phase III NETTER-1 trial. *European Journal of Cancer* **51** S710. (doi:10.1016/S0959-8049(16)31929-3)

Strosberg JR, Cives M, Hwang J, Weber T, Nickerson M, Atreya CE, Venook A, Kelley RK, Valone T, Morse B, *et al.* 2016. A phase II study of axitinib in advanced neuroendocrine tumors. *Endocrine-Related Cancer* **23** 411–418. (doi:10.1530/ERC-16-0008)

Svejda B, Kidd M, Kazberouk A, Lawrence B, Pfragner R & Modlin IM 2011 Limitations in small intestinal neuroendocrine tumor therapy by mTor kinase inhibition reflect growth factor-mediated PI3K feedback loop activation via ERK1/2 and AKT. *Cancer* **117** 4141–4154. (doi:10.1002/cncr.26011)



http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0370



Published by Bioscientifica Ltd.

Tamburrino D, Spoletini G, Partelli S, Muffatti F, Adamenko O, Crippa S & Falconi M 2016 Surgical management of neuroendocrine tumors. *Best Practice & Research: Clinical Endocrinology & Metabolism* **30** 93–102. (doi:10.1016/j.beem.2015.10.003)

Tang LH, Contractor T, Clausen R, Klimstra DS, Du YC, Allen PJ, Brennan MF, Levine AJ & Harris CR 2012 Attenuation of the retinoblastoma pathway in pancreatic neuroendocrine tumors due to increased cdk4/cdk6. *Clinical Cancer Research* **18** 4612–4620. (doi:10.1158/1078-0432.CCR-11-3264)

Tang LH, Basturk O, Sue JJ & Klimstra DS 2016 A practical approach to the classification of WHO grade 3 (G3) well-differentiated neuroendocrine tumor (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC) of the pancreas. *American Journal of Surgical Pathology* **40** 1192–1202. (doi:10.1097/PAS.0000000000000662)

van Essen M, Sundin A, Krenning EP & Kwekkeboom DJ 2014 Neuroendocrine tumours: the role of imaging for diagnosis and therapy. *Nature Review Endocrinology* **10** 102–114. (doi:10.1038/nrendo.2013.246)

Vandamme T, Beyens M, de Beeck KO, Dogan F, van Koetsveld PM, Pauwels P, Mortier G, Vangestel C, de Herder W, Van Camp G, *et al.* 2016 Long-term acquired everolimus resistance in pancreatic neuroendocrine tumours can be overcome with novel PI3K-AKT-mTOR inhibitors. *British Journal of Cancer* **114** 650–665. (doi:10.1038/bjc.2016.25)

Vijayvergia N, Boland PM, Handorf E, Gustafson KS, Gong Y, Cooper HS, Sheriff F, Astsaturov I, Cohen SJ & Engstrom PF 2016 Molecular profiling of neuroendocrine malignancies to identify prognostic and therapeutic markers: a Fox Chase Cancer Center Pilot Study. *British Journal of Cancer* **115** 564–570. (doi:10.1038/bjc.2016.229)

Vinik A, Van Cutsem E, Niccoli P, Raoul JL, Bang YJ, Borbath I, Valle JW, Metrakos P, Smith D, Chen JS, *et al.* 2012 Updated results from a phase III trial of sunitinib versus placebo in patients with progressive, unresectable, well-differentiated pancreatic neuroendocrine tumor (NET). *Journal of Clinical Oncology* **30** (Supplement) abstract 4118. (available at: http://meeting.ascopubs.org/cgi/content/abstract/30/15_suppl/4118)

von Marschall Z, Scholz A, Cramer T, Schäfer G, Schirner M, Oberg K, Wiedenmann B, Höcker M & Rosewicz S 2003 Effects of interferon alpha on vascular endothelial growth factor gene transcription and tumor angiogenesis. *Journal of the National Cancer Institute* **95** 437–448. (doi:10.1093/jnci/95.6.437)

Walter T, van Brakel B, Vercherat C, Hervieu V, Forestier J, Chayvialle JA, Molin Y, Lombard-Bohas C, Joly MO, *et al.* 2015 O6-methylguanine-DNA methyltransferase status in neuroendocrine tumours: prognostic relevance and association with response to alkylating agents. *British Journal of Cancer* **112** 523–531. (doi:10.1038/bjc.2014.660)

Wheler JJ, Janku F, Naing A, Li Y, Stephen B, Zinner R, Subbiah V, Fu S, Karp D, Falchook GS, *et al.* 2016 TP53 Alterations correlate with response to VEGF/VEGFR inhibitors: implications for targeted therapeutics. *Molecular Cancer Therapy* **15** 2475–2485. (doi:10.1158/1535-7163.MCT-16-0196)

Wolin EM, Jarzab B, Eriksson B, Walter T, Toumpanakis C, Morse MA, Tomassetti P, Weber MM, Fogelman DR, Ramage J, *et al* 2015 Phase III study of pasireotide long-acting release in patients with metastatic neuroendocrine tumors and carcinoid symptoms refractory to available somatostatin analogues. *Drug Design, Development and Therapy* **9** 5075–5086. (doi:10.2147/DDDT)

Xu J, Pham CG, Albanese SK, Dong Y, Oyama T, Lee CH, Rodrik-Outmezguine V, Yao Z, Han S, Chen D, *et al.* 2016 Mechanistically distinct cancer-associated mTOR activation clusters predict sensitivity to rapamycin. *Journal of Clinical Investigation* **126** 3526–3540. (doi:10.1172/JCI86120)

Yao JC & Phan A 2011 Overcoming antiangiogenic resistance. *Clinical Cancer Research* **17** 5217–5219. (doi:10.1158/1078-0432.CCR-11-1219)

Yao JC, Zhang JX, Rashid A, Yeung SC, Szklaruk J, Hess K, Xie K, Ellis L, Abbruzzese JL & Ajani JA 2007 Clinical and in vitro studies of imatinib in advanced carinoid tumors. *Clinical Cancer Research* **13** 234–240. (doi:10.1158/1078-0432.CCR-06-1618)

Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, *et al.* 2008*a* One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of Clinical Oncology* **26** 3063–3072. (doi:10.1200/JCO.2007.15.4377)

Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, *et al.* 2008*b* Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *Journal of Clinical Oncology* **26** 4311–4318. (doi:10.1200/JCO.2008.16.7858)

Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, Cherfi A & Öberg KE 2011*a* Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *Journal of Clinical Endocrinology and Metabolism* **96** 3741–3749. (doi:10.1210/jc.2011-0666)

Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, *et al.* 2011*b* Everolimus for advanced pancreatic neuroendocrine tumors. *New England Journal of Medicine* **364** 514–523. (doi:10.1056/NEJMoa1009290)

Yao J, Guthrie K, Moran C, Strosberg J, Kulke M, Chan J, LoConte NK, McWilliams RR, Wolin E, Mattar BI, *et al.* 2015 Phase III prospective randomized comparison of depot octreotide plus interferon alpha-2b versus depot octreotide plus bevacizumab (NSC #704865) in advanced, poor prognosis carcinoid patients (NCT00569127). *Journal of Clinical Oncology* **33** (Supplement) abstract 4004. (available at: http://meeting.ascopubs.org/cgi/content/abstract/33/15_suppl/4004)

Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, Tomasek J, Raderer M, Lahner H, Voi M, *et al.* 2016 RAD001 in advanced neuroendocrine tumours, Fourth Trial (RADIANT-4) Study Group. Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet* **387** 968-977. (doi:10.1016/S0140-6736(15)00817-X)

Yu D, Leja-Jarblad J, Loskog A, Hellman P, Giandomenico V, Oberg K & Essand M 2016 Preclinical evaluation of advince, an oncolytic adenovirus adapted for treatment of liver metastases from neuroendocrine cancer. *Neuroendocrinology* [in press]. (doi:10.1159/000448430)

Yuan F, Shi M, Ji J, Shi H, Zhou C, Yu Y, Liu B, Zhu Z & Zhang J 2014 KRAS and DAXX/ATRX gene mutations are correlated with the clinicopathological features, advanced diseases, and poor prognosis in Chinese patients with pancreatic neuroendocrine tumors. *International Journal of Biological Sciences* **10** 957–965. (doi:10.7150/ijbs.9773)

Zurita AJ, Khajavi M, Wu HK, Tye L, Huang X, Kulke MH, Lenz HJ, Meropol NJ, Carley W, DePrimo SE, *et al.* 2015 Circulating cytokines and monocyte subpopulations as biomarkers of outcome and biological activity in sunitinib-treated patients with advanced neuroendocrine tumours. *British Journal of Cancer* **112** 1199–1205. (doi:10.1038/bjc.2015.73)

Received in final form 19 September 2016
Accepted 20 September 2016
Accepted Preprint published online 20 September 2016





Published by Bioscientifica Ltd.