Hitting the target: where do molecularly targeted therapies fit in the treatment scheduling of neuroendocrine tumours?

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Abstract

Neuroendocrine tumours (NETs) are a rare and heterogeneous group of tumours whose incidence is increasing and their prevalence is now greater than that of any other upper gastrointestinal tumour. Diagnosis can be challenging, and up to 25% of patients present with metastatic disease. Following the recent FDA approval of two new molecularly targeted therapies for the treatment of advanced pancreatic NETs (pNETs), the first in 25 years, we review all systemic therapies and suggest where these newer targeted therapies fit in the treatment schedule for these challenging tumours. Clinical trial data relating to the routine use of sunitinib and everolimus in low–intermediate-grade pNETs are summarised alongside newer molecularly targeted agents undergoing clinical assessment in NETs. We particularly focus on the challenge of optimal scheduling of molecularly targeted treatments around existing systemic and localised treatment such as chemotherapy or radiotargeted therapy. We also discuss application of current evidence to subgroups of patients who have not so far been directly addressed such as those with poorer performance status or patients receiving radical surgery who may benefit from adjuvant treatment.

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Introduction

Neuroendocrine tumours (NETs) comprise a genetically and clinically heterogeneous group of tumours. In order to maximise the best clinical outcome for patients, they should be managed by a multidisciplinary group of experienced clinicians including oncologists, gastroenterologists, endocrinologists, nuclear medicine physicians and surgeons. NETs can present with a variety of constitutional symptoms, and diagnosis can be delayed for up to 5 years on average, particularly if non-syndromic. Prognostication and clinical outcomes are currently based on the primary site of the NET and the histological grade of the tumour. Radical surgery for localised disease is the only known curative treatment. Systemic therapies include somatostatin analogues (SSAs), chemotherapy, peptide receptor radionuclide therapy (PRRT),

interferon- α (IFN α) and most recently the molecularly targeted agents sunitinib and everolimus. It is clear that marked differences in molecular pathogenesis are evident between NETs of different sites of origin. This has been observed in clinical trials of NETs in recent years where marked differences in response to molecularly targeted therapy had been observed in pancreatic, bronchial and intestinal NETs. It is now widely accepted that pathological subgroups of NET (depending on site of origin) should be assessed while developing clinical trials (Kulke *et al.* 2011).

In this review, we summarise the current systemic treatment options for NETs of bronchial, pancreatic and intestinal origin and discuss recent advances in the elucidation of the biological pathways involved in the development of pancreatic NETs (pNETs), focusing on the use and suggested scheduling of sunitinib and everolimus.

Pathogenesis

NETs are 'rare' tumours with a relatively low incidence (5/100 000 population), and high prevalence, higher than any other upper gastrointestinal cancer (Yao *et al.* 2008a,b), both the incidence and prevalence are rising. Over 90% of NETs are sporadic, the remaining ~10% are associated with familial syndromes including multiple endocrine neoplasia 1 (MEN1) syndrome, von Hippel–Lindau (VHL), tuberous sclerosis (TSC) and neurofibromatosis (NF1) (Calender 2006). Current pathological staging and grading differs between Europe and the USA; however, both the classification systems are centred around the primary site of the tumour and histological grade. In Europe, the Ki-67 proliferative index is used to differentiate tumours of low (<2%), intermediate (2–20%) and high (>20%) grades, whereas in the USA, tumours are graded as 'well' and 'poorly' differentiated where 'well' equates to low–intermediate-grade and 'poorly' equates to high-grade tumours. The current staging systems are reviewed by Kulke *et al.* (2011). The most common familial NET syndromes are summarised below.

Familial NET syndromes

MEN1 syndrome is characterised by parathyroid hyperplasia/adenoma, pancreatic endocrine tumours (25–80% mostly non-functioning, gastrinomas or insulinomas), pituitary tumours and adrenocortical adenomas (Brandi *et al.* 2001, Akerstrom *et al.* 2005). MEN1 is the result of germline inactivation of the tumour suppressor *MEN1* gene (localised to 11q13) and subsequent loss of the wild-type allele (Marx 2005).

VHL syndrome is caused by an autosomal dominantly inherited germline mutation in the *VHL* tumour suppressor gene (at locus 3p25.5), the second wild-type allele is lost somatically. Pancreatic non-functioning NETs develop in 12–17% of patients with VHL (Hammel *et al.* 2000, Blansfield *et al.* 2007). The syndrome leads to the development of central nervous system and retinal haemangiomas, renal carcinomas and pheochromocytomas and pNETs (Lott *et al.* 2002).

NF1 is an autosomal dominant disorder characterised by café au lait spots, neurofibromas, optic gliomas, iris hamartomas and pancreatic somatostatinomas (6%). This syndrome is caused by mutation of the *NF1* tumour suppressor gene (chromosome 17q11.2). It has been shown that *NF1* acts as a negative regulator of mammalian target of rapamycin (mTOR; Johannessen *et al.* 2005), suggesting that NF1-related NETs might be particularly susceptible to mTOR pathway-targeted therapies.

TSC is an autosomal dominant disease characterised by hamartomas and astrocytomas and well-differentiated tumours in brain, heart, skin, kidney, lung and pancreas (NET <5%), and it is often associated with epilepsy and learning difficulties. TSC is caused by mutation of *TSC1* (hamartin, chromosome 9q34) or *TSC2* (tuberin, chromosome 16p13.3). The hamartin–tuberin complex normally inactivates the protein Ras homologue enriched in brain (Rheb) that functions to activate the mTOR signalling pathway (Huang & Manning 2008). The TSC protein complex therefore acts as an intrinsic suppressor of mTOR signalling, and when mutated leads to constitutive activation, again suggesting susceptibility to mTOR inhibition therapy in these patients.

Signalling pathways involved in NET development

Vascular endothelial growth factor (VEGF) pathway activation plays a major role in the development of several solid cancers. Activation of the VEGF receptor (VEGFR) family triggers a signalling cascade resulting in increased endothelial cell survival, mitogenesis, migration and vascular permeability. Associated pathways include induction of the phosphoinositide 3-kinase/AKT (PI3K/AKT) pathway, metalloproteinases, activation of mitogen-activated protein kinases and upregulation of integrin expression (Hicklin & Ellis 2005). VEGF is physiologically upregulated by hypoxia inducible factor (HIF1- α (HIF1A)), which is usually degraded in the presence of VHL. In the absence or mutation of *VHL*, levels of HIF-1 α rise leading to the transcription of over 100 hypoxia-related genes involving angiogenesis, glucose metabolism, erythropoietin production, cellular proliferation, tumour invasion/metastasis and *p53* (*TP53*)-mediated apoptosis (Semenza 2003). Bi-allelic inactivation of *VHL* leads to the development of pNET development in up to 12% of cases (Hammel *et al.* 2000). There is increasing evidence to suggest that disruption of the VHL pathway and subsequent HIF activation occurs in sporadic pNETs (Couvelard *et al.* 2005, 2008, Schmitt *et al.* 2009).

Dysregulation of the mTOR pathway is known to be involved in the pathogenesis of up to 80% of human cancers (Watanabe *et al.* 2011). It forms two complexes (mTORC1 and 2) and integrates signals from multiple sources (including glucose, growth factors such as insulin-like growth factor 1 (IGF1) and hormones such as leptin) by signalling through the PI3K/AKT system (Goldstein & Meyer 2011). Through phosphorylation of a variety of kinases and

proteins, mTOR regulates survival, metabolism, proliferation and angiogenesis (the latter through activation of HIF). Inhibition of this pathway by everolimus and similar compounds is therefore an attractive anti-cancer mechanism. Figure 1 shows an overview of intracellular signalling pathways.

Following the recent exome sequencing of sporadic pNETs, significant advances have been made in determining the biological pathways underlying the pathogenesis of pNETs (Jiao *et al.* 2011). This study on 68 (ten discovery and 58 validation) primary and metastatic sporadic pNETs identified mutations in *ATRX/DAXX* and *menin* in 43 and 44% of tumours respectively. All these genes are involved in chromatin modelling, and the functional implications of these mutations are yet to be elucidated. Patients harbouring these mutations were found to have a significantly improved overall survival. Fourteen per cent of pNETs were also found to harbour mutations in genes involved in the mTOR pathway including phosphatase and

tensin homologue (*PTEN*), *TSC2* and PI3K catalytic alpha polypeptide (*PIK3CA*; Jiao *et al.* 2011). A previous study found that 85% of pNETs had reduced expression of *TSC2* or *PTEN* or both, these tumours were more aggressive and found to have a higher Ki-67 proliferation index, shorter time to progression and overall survival (Missiaglia *et al.* 2010). These two studies suggest that there is an identifiable cohort of patients who would gain particular benefit from treatment targeted to inhibiting the mTOR pathway; however, these potential novel biomarkers require further validation in larger clinical cohorts.

It is widely accepted that in order to fully understand the pathogenesis and clinically stratify this heterogeneous group of tumours, comprehensive genomic analyses are required and are currently ongoing.

The rationale and preclinical data supporting the use of VEGF and mTOR molecularly targeted therapies are discussed in the Molecularly Targeted Therapy section of this review.

Figure 1 Intracellular signalling pathways in neuroendocrine tumours showing the PI3K/AKT/mTOR; RAS/RAF/MEK/ERK; PLC/PKC and JAK/STAT pathways. Adapted from Raymond *et al.* (2011a,b). VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet derived growth factor receptor; SSR, somatostatin receptor; IGF1R, insulin like growth factor 1 receptor; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma protein; PLC, phospholipase C; PKC, protein kinase C; JAK/STAT, Janus kinase signal transducer and activator of transcription.

Review of systemic therapies and current guidelines

Due to the rarity of NETs, there is a lack of large-scale randomised controlled trials (RCTs); therefore, much of our understanding in NET management is based on non-randomised phase II clinical studies that have not compared the treatment under investigation against any best supportive or gold standard therapy. In light of this, treatment protocols and sequencing of therapies vary from centre to centre, although national and international guidelines have been published. An overview of available treatment options for gastroenteropancreatic (GEP) NETs is shown in Fig. 2.

There have been four RCTs of chemotherapy, two in pNETs and two in gastrointestinal NETs, three for IFN and three studying molecularly targeted therapies (summarised in Table 1). No RCTs have been performed in the field of PRRT. The first novel therapies to be licensed for NET for 25 years were introduced in 2011: sunitinib and everolimus. Both are molecularly targeted agents inhibiting well-described

cancer signalling pathways (with everolimus targeting mTOR and sunitinib VEGF). This represents significant progress for NET systemic therapy not only in improved clinical outcome for patients but also through demonstrating the feasibility of performing large-scale international randomised controlled phase III trials in rare cancers.

The most recently published European Neuroendocrine Tumour Society (ENETS) guidelines discuss the use of molecularly targeted treatment in the second- or third-line setting for well-differentiated non-functioning pancreatic tumours, and everolimus for gastrointestinal tumours (Falconi et al. 2011, Pavel et al. 2011a,b). The most recent North American NET Society (NANETS) guidelines only briefly discuss targeted therapy and provide no guidance on usage (Kulke et al. 2010). ESMO guidelines on GEP NETs provide no specific guidance on the subject of molecularly targeted therapy, reflecting the rapid progress and new developments over the last year (Oberg et al. 2010a,b). The United Kingdom and Ireland NET Society (UKINETS) group have

Figure 2 Flow diagram of therapies for localised and advanced gastroenteropancreatic neuroendocrine tumours.

Table 1 Randomised controlled trials in NETs

Reference	Trial summary	Number of participants	Outcome
Moertel <i>et al.</i> (1980)	Chemotherapy STZ vs F-STZ in islet cell	84	ORR 63 vs 36% CR 33 vs 12%
Moertel <i>et al.</i> (1992)	Chemotherapy STZ-dox vs STZ-F vs chlorozotocin in islet cell	105	ORR 69 vs 45% OS 2.2 vs 1.4 years (STZ-dox vs STZ-F)
Engstrom <i>et al.</i> (1984)	Chemotherapy STZ-F vs dox in carcinoid	172	ORR 22 vs 21% OS 64 vs 48 weeks
Sun <i>et al.</i> (2005)	Chemotherapy F-Dox vs F-STZ vs DTIC in carcinoid	249	ORR 15.9 vs 16% OS 24.3 vs 15.7m (F-STZ vs F-Dox)
Kolby <i>et al.</i> (2003)	IFN SSA+IFN vs SSA in midgut carcinoid	68	5 years OS: 57 vs 37% ($P=0.13$)
Faiss <i>et al.</i> (2003)	IFN SSA vs IFN vs combination in GEP NET	83	Comparable antiproliferative effects
Arnold <i>et al.</i> (2005)	IFN Octreotide vs Octreotide+IFN in GEP NET	109	Combination therapy not superior to monotherapy for PFS or OS
Raymond <i>et al.</i> (2011a,b)	Targeted Sunitinib vs BSC in pNET	171	PFS 11.4 vs 5.5m ORR 9.3 vs 0%
Yao <i>et al.</i> (2011)	Targeted Everolimus vs placebo in advanced low/ intermediate grade pNET	410	PFS 11.0 vs 4.6m
Pavel <i>et al.</i> (2011a,b)	Targeted Everolimus plus SSA vs SSA in NET associated with carcinoid syndrome	429	PFS 16.4m vs 11.3 ($P=0.026$)

STZ, streptozocin; F, fluorouracil; dox, doxorubicin; DTIC, dacarbazine; IFN, interferon; SSA, somatostatin analogue; GEP NET, gastroenteropancreatic neuroendocrine tumour; BSC, best supportive care; pNET, pancreatic NET.

published the second most recent guidelines for the management of GEP NETs which includes the use of molecularly targeted therapy (Ramage *et al.* 2012).

Chemotherapy

It was first noted in the 1970s and 80's that streptozocin, doxorubicin and 5-fluorouracil (5-FU) have clinical benefit in NETs (Strosberg *et al.* 2011a,b). Since then there have been a number of phase II trials which have failed to replicate the initial efficacy of streptozocin and doxorubicin originally reported for these agents (50% – streptozocin, 45% – 5-FU/doxorubicin). The original trials used non-radiological parameters for response such as clinical examination for hepatomegaly and serum tumour marker levels rather than standardised radiological criteria such as Response Evaluation Criteria in Solid Tumours (RECIST). Three studies since (using RECIST criteria) have yielded similar results using combination therapy with streptozocin, 5-FU and doxorubicin (Kouvaraki *et al.* 2004), streptozocin, cisplatin and 5-FU (Turner *et al.* 2010), streptozocin and doxorubicin (Delaunoy *et al.* 2004). The partial response (PR) rates for these studies being 39, 38 and 36% respectively. The observed response rate (RR) varies between 30 and 40% for combination therapy in

the majority of studies, with one recent study reporting a response rate of 70% (Strosberg *et al.* 2011a,b). pNETs are more sensitive than non-pNETs to streptozocin based combination chemotherapy, this is supported by *in vitro* evidence which suggests that streptozocin is preferentially taken up by β -islet cells in the pancreas (Anderson *et al.* 1974). High-grade NETs (Ki-67 > 20%) have ~50% response rate to platinum based regimes. Newer chemotherapy agents such as gemcitabine and docetaxel have been trialled in the phase II setting and do not appear promising clinically.

Most recently, temozolomide has been trialled with capecitabine (an oral pro-drug of 5-FU) in the first line treatment of low- and intermediate-grade pNETs. Although non-randomised and retrospective, this trial of only 30 patients found a remarkable response rate of 70% (Strosberg *et al.* 2011a,b) with progression-free survival of 18 months and 2 year overall survival of 92%. Previously, this regimen has been used as second-line treatment where streptozocin based therapy had failed in intermediate and high-grade tumours (Isacoff *et al.* 2006). A phase II trial of temozolomide in combination with thalidomide found a radiological response rate of 25% (45% in pNETs and only 7% in gastrointestinal tumours; Kulke *et al.* 2006).

The choice of chemotherapy regime depends on the grade (based on Ki-67 proliferative index), primary site of the tumour and stage of disease. Other factors such as performance status (PS) and previous treatment schedules require consideration on a case-by-case basis by the multidisciplinary team. Combination chemotherapy with up to three drugs including streptozocin, doxorubicin, 5-FU (or capecitabine), temozolomide, cisplatin and etoposide is commonly used, the exact schedule and dosing varies from centre to centre. The ENETS guidelines for standard of care with systemic chemotherapy have most recently been published in 2011 (Pavel *et al.* 2011a,b).

Low- to intermediate-grade (well-differentiated) PNETs and gastrointestinal NETs

pNETs are known to have a higher response rate to streptozocin-based chemotherapy; therefore in treatment of in-operable, metastatic tumours, chemotherapy is recommended following failure of biotherapy. The commonly used combination chemotherapy regimens lead to an expected response rate of between 30 and 40%. Our institute uses a combination of 5-FU, streptozocin and cisplatin given intravenously 3 weekly as an outpatient. This regime is well tolerated, given as an outpatient and unlike regimes containing doxorubicin, patients are not exposed to associated cardiotoxicity and do not experience alopecia. A review of 82 patients treated with this regime found PR and stable disease (SD) in 38 and 51% of PNET patients and PR and SD of 25 and 50% of non-pNET patients respectively (Turner *et al.* 2010).

Gastrointestinal NETs have a lower response rate to chemotherapy, and it is recommended that chemotherapy is used as a second-line therapy where other systemic therapy has failed unless the tumour is of high grade (Ki-67 > 20%) or if the tumour is of goblet cell origin. Metastatic goblet cell tumours arise almost exclusively in the appendix, many of the markers they express are the same as colorectal adenocarcinoma; however, the chromosomal changes occurring in these tumours are often the same as ileal NETs. Currently, they are treated with the same chemotherapy agents as colorectal adenocarcinoma – 5-FU and oxaliplatin (FOLFOX), given intravenously 2 weekly. This regimen is also given in the adjuvant setting for patients with stage III disease post-operatively. Recent ENETS Consensus guidelines for goblet cell carcinomas review current standards of care (Pape *et al.* 2011).

High-grade (poorly differentiated) NETs

Platinum-based chemotherapy is recommended for high-grade (Ki-67 > 20%) tumours. When used in combination with etoposide, a response rate of ~50% is observed (Eriksson *et al.* 2009). In high-grade PNETs with multiple metastases, chemotherapy is advised as the first-line therapy. Response to treatment is assessed by RECIST criteria on review of cross-sectional computed tomography (CT) imaging 2–3 months after commencing therapy, tumour markers (where available) and clinical response – in terms of the patient symptoms.

In patients with low–intermediate-grade tumours, it is common to observe a delayed response to treatment on CT imaging 4 months after commencing therapy (Kouvaraki *et al.* 2004, Turner *et al.* 2010). Therefore, if SD is present at the first-interval scan, the chemotherapy course should be completed if the patient is able to tolerate the treatment and there are no other complications.

Bronchial NETs

A small study on single-agent temozolomide reported a clinical benefit rate (response or stabilisation) in 62% of participating patients with bronchial NET ($n=13$; Ekeblad *et al.* 2007). In two cases, neoadjuvant 5-FU/cisplatin/streptozocin chemotherapy has been used successfully to downstage bronchial NET before curative surgery (Srirajaskanthan *et al.* 2009), other small series reported promising results with doxorubicin-based chemotherapy (Granberg *et al.* 2001). Prospective randomised trials are required to determine the role of chemotherapy and optimal treatment regimen in these patients. Clinical practice guidelines for bronchial NET have been published by the European Society of Medical Oncology (Oberg *et al.* 2010a,b).

Immunological modulators

IFNs have been used in cancer therapy for over 25 years and are known to exert anti-tumoral effects through stimulation of T cells, induction of cell cycle arrest and inhibition of angiogenesis (Parmar & Plataniias 2005). Interestingly, in NETs, IFNs can upregulate somatostatin receptor expression (Hofland *et al.* 1999). IFN α is the IFN subtype most studied in NETs both *in vitro* and clinically. The first clinical study on IFN α in NETs was conducted in 1983 in patients with midgut NETs (Oberg *et al.* 1983).

To date, however, there have been promising phase II studies, but all the three RCTs published have been

non-significant when IFN α was trialled concomitantly with SSAs in intestinal NETs. In one such study in which 68 patients were treated with subcutaneous octreotide alone or in combination with 3 million units of IFN α five times per week, the 5-year overall survival was increased in the combination arm (57 vs 37%) but was not found to be significant ($P=0.13$; Kolby *et al.* 2003). Objective response rates in two further RCTs were ~ 7 and 6% respectively (Faiss *et al.* 1996, Arnold *et al.* 2005). All the three RCTs performed to date were underpowered and no statistically significant overall survival data can be determined. Alongside this, an optimum dosing schedule is yet to be published. Many clinicians chose not to use IFN α due to the difficulties of balancing the sometimes debilitating side effects of fatigue, myalgia and fever; bone marrow suppression and depression with clinical benefit.

In selected cases in centres with previous clinical experience, however, IFN α can lead to symptom control and disease stabilisation of long-term duration up to a number of years.

Somatostatin analogues

Up to 80% of GEP NETs express somatostatin receptors (SST $_2$ and SST $_5$ primarily). SSAs have been used for the treatment of NETs since the 1980s and have a marked effect on improving many of the hormonal symptoms experienced in functioning NETs. Physiologically, somatostatin causes reduced gastro-intestinal secretion, inhibition of peristalsis, decreased portal blood flow and down-regulation of the secretion of several intestinal hormones.

Somatostatin binds to five G-protein-coupled somatostatin receptors (SSTR1–5). Initial clinical use of human somatostatin was hindered by its short half-life of ~ 2 min. The key study that demonstrated a clinical effect of SSAs was published by Kvolts *et al.* (1986); this demonstrated an improvement in flushing and diarrhoea in 22/25 (88%) patients, with a significant reduction in urinary 5-hydroxyindoleacetic acid (5-HIAA) in 18/25 (72%) patients. More recently, long-acting depot synthetic SSAs have been manufactured, which have been used for symptom control including octreotide acetate (Rubin *et al.* 1999) and lanreotide (Ruszniewski *et al.* 1996). The PROMID study in 2009 (Rinke *et al.* 2009) demonstrated improved time to progression and disease control in intestinal NETs with SSA use (TTP 14.3m with octreotide LAR vs 6m with placebo $P<0.0001$). Since then, several trials have confirmed the anti-secretory effects of SSAs in functional intestinal NETs

as well as VIPoma and glucagonoma (Maton *et al.* 1989, di Bartolomeo *et al.* 1996, Rubin *et al.* 1999, O'Toole *et al.* 2000). There is also evidence of an anti-proliferative effect of SSAs on type I and II gastric carcinoids with reduction in tumour volume and reduced serum gastrin levels demonstrated (Tomassetti *et al.* 2000, Grozinsky-Glasberg *et al.* 2008a,b).

The most commonly used long-acting SSAs in clinical use are octreotide LAR (10–30 mg given by deep i.m. injection 3–4 weekly) and lanreotide (60–120 mg given s.c. every 4–6 weeks). Both analogues bind with high avidity to SST $_2$ and SST $_5$. In patients who develop refractory hormonal symptoms, the dose of both these agents can be increased by increasing the frequency of injections to 3 weekly (O'Toole *et al.* 2000). Current recommendations for baseline investigations and safety monitoring have been produced by ENETS (Oberg *et al.* 2009), and an up to date review of the role of SSAs has been recently published (Khan & Caplin 2011).

There are several ongoing trials with pasireotide (SOM230, a novel multitargeted SSA that binds SSTR1–4) for patients with refractory symptoms and palliation for flushing and diarrhoea in patients who are resistant to treatment with octreotide, both as a single agent and in combination with agents such as everolimus (www.clinicaltrials.gov, NCT01364415 Dose escalation study of pasireotide (SOM230) in patients with advanced NETs. (cited 2011), www.clinicaltrials.gov, NCT01263353 Safety and tolerability of pasireotide LAR in combination with everolimus in advanced metastatic NETs (COOPERATE-1). (cited 2011)).

PRRT with radiolabelled SSAs (PRRT)

PRRT has been used in the treatment of NETs since the early 1990s. The majority of clinical trial data available are from non-randomised retrospective case series; to date, no RCTs have been performed with PRRT. Due to variation in patient selection, dosing, scheduling and total number of treatments, it can be challenging to draw firm conclusions from the literature. There does appear, however, to be a benefit for selected patients with response rates in the range of 40% (Oberg *et al.* 2009). Early trials with ^{111}In -pentetreotide labelled with somatostatin (the isotope used for Octreoscan) at high cytotoxic doses lead to clinical benefit in only 7% (Krenning *et al.* 1993) to 19% (Buscombe *et al.* 2003) of patients, this was most likely due to the low tissue penetration of the emitted Auger electrons from the ^{111}In isotope (Krenning *et al.* 1993, Strosberg *et al.* 2011a,b). Today, the most commonly used isotopes are ^{90}Y and more recently ^{177}Lu .

The use of PRRT is recommended for selected metastatic GEP NET patients in specialist centres with expertise and legal permission to manufacture and safely deliver radiolabelled isotopes. In order to be eligible, the patients' tumour should demonstrate at least as high uptake as normal liver on Octreoscan (^{111}In -pentetreotide). At this time, there are no consistent guidelines regarding optimum dose, cycle interval or safe cumulative dose for these therapies (Kwekkeboom *et al.* 2009). Treatment schemes and isotopes currently used depend on local expertise, experience and clinical judgement.

^{90}Y trium-labelled PRRT

^{90}Y trium is a high-energy β -particle emitter that has a maximum tissue penetration of 12 mm. There are two ^{90}Y trium-based isotopes in clinical use – [^{90}Y -DOTA-Tyr³-octreotide] (^{90}Y -DOTATOC) and [^{90}Y -DOTA(0)-D-Phe¹-Tyr³-octreotate] (^{90}Y -DOTATATE).

The largest study to date using ^{90}Y -DOTATOC including 1109 patients found a radiological response (on cross-sectional imaging) in 34.1% and clinical response in 29.7%. Patients that responded were found to have longer median survival (44.7 vs 18.3 months). The degree of uptake on the pretreatment Octreoscan was found to be predictive of response to treatment and overall survival (Imhof *et al.* 2011).

^{90}Y trium-labelled lanreotide has also been trialled as it is thought to have higher affinity for SST₃ and SST₄ than octreotide. However, when used clinically, it was found to have lower affinity for SST₂ than ^{90}Y -DOTATOC, a response rate of 21% was observed in a single study of 39 GEP patients (Virgolini *et al.* 2002). A phase II study of ^{90}Y -DOTATATE in 60 GEP NET patients found a PR rate of 23% with SD observed in the remainder of patients (Cwikla *et al.* 2010). At 6 months of follow-up, a clinical PR was observed in 72% of patients. The median overall survival in this patient cohort was 22 months.

Treatment with ^{90}Y -DOTATOC and ^{90}Y -DOTATATE is generally well tolerated with the most common side effects of nausea and vomiting being caused by the administration of amino acid solutions. Reversible bone marrow suppression is seen in up to 43% and reversible nephrotoxicity in 10% of patients (Oberberg *et al.* 2009), rarely myelodysplastic syndrome (2%) or acute leukaemia may occur.

^{177}Lu tetium-labelled PRRT

^{177}Lu tetium-octreotate [^{177}Lu -DOTA⁰,Tyr³] is a β - and γ -emitting compound that has nine times greater affinity for SST₂ than ^{177}Lu tetium-octreotide.

Moreover, tumour uptake is four to five times higher than that seen in normal renal and hepatic tissue. It has lower tissue penetration than ^{90}Y trium, making it a desirable isotope for the treatment of smaller tumours/metastases. Response rates of up to 30% and median time to progression of 40 months have been observed in a study of 310 patients (Kwekkeboom *et al.* 2008). Again, the degree of uptake in the pretreatment Octreoscan was indicative of response to treatment. ^{177}Lu has the benefit of enabling post-therapy imaging demonstrating localisation of therapy due to its γ -emissions. For all PRRTs, the longer term toxicity to bone marrow and kidneys needs to be evaluated.

Molecularly targeted therapy

A number of molecularly targeted agents have been trialled in NETs over the last 10 years, including monoclonal antibodies against EGFR, VEGF, IGF1R and more recently oral tyrosine kinase inhibitors. Initial studies did not routinely stratify patient selection based on the primary site of the tumour and this lead to inconclusive trial outcomes (Faivre *et al.* 2010). Therefore, patient stratification by primary tumour site and histological grade is key when designing future trials in NETs.

Pancreatic NETs

VEGF- and mTOR-targeted therapies

It is well documented that NETs are highly vascularised, and immunohistochemistry studies demonstrate that VEGF and HIF activation markers are over-expressed in pNETs (Zhang *et al.* 2007, Pape *et al.* 2011). Secretion of VEGF is inhibited by octreotide and inhibitors of the PI3K/AKT/mTOR pathway (Villaume *et al.* 2010), suggesting alternative mechanisms of action of these therapies. Initial clinical trials investigating anti-VEGF-related therapy included the use of sorafenib and bevacizumab. More recently, sunitinib has demonstrated improved progression free survival (PFS) in a large phase III RCT when used selectively in pNETs (Raymond *et al.* 2011a,b).

Sorafenib is a multiple kinase inhibitor affecting VEGFR2, PDGFR (PDGFRB), FGFR1 and FLT3. It was shown to have modest activity in NETs in a phase II study led by Hobday *et al.* (2007) in which objective response rate (ORR) was 7–11% in non-selected pancreatic and intestinal NETs. Bevacizumab (an anti-VEGF monoclonal antibody) demonstrated anti-tumoral activity in mouse models where it inhibited

tumour angiogenesis and impaired tumour growth (Zhang *et al.* 2007). However, when trialled clinically alongside SSAs, IFN- α (Yao *et al.* 2008a,b) and chemotherapy in a phase II trial, improved PFS was observed in the bevacizumab monotherapy arm when compared with IFN α monotherapy. There is now a ongoing phase III trial comparing the PFS of long-acting octreotide given concomitantly with either bevacizumab or IFN α -2b (www.clinicaltrials.gov. NCT00569127 Octreotide and IFN alfa-2b or bevacizumab in treating patients with metastatic or locally advanced, high risk neuroendocrine tumor. (cited 2011)).

In 2011, two pivotal large-scale RCTs were published, which demonstrated an improved progression-free survival in the use of sunitinib (Raymond *et al.* 2011a,b) and everolimus (Yao *et al.* 2011) in the treatment of selected patients with low–intermediate-grade pNETs. The early phase and phase III trial outcomes are discussed below.

Sunitinib

Sunitinib is a multiple kinase inhibitor that targets VEGFRs 1–3, platelet-derived growth factor (PDGF) receptor α (PGDFA) and β (PGDFA), c-kit, RET and FLT3. It is used in clear cell renal cancers and gastrointestinal stromal tumours as second-line therapy following the failure of imatinib. Preclinical studies on the RIP-Tag2 mouse model of pNETs found that sunitinib decreased tumour mass and inhibited VEGFR-dependent endothelial cell proliferation (Raymond *et al.* 2009).

The first clinical efficacy of sunitinib in NETs was observed in a phase I study that included three NET patients (Faivre *et al.* 2007); this was quickly followed by phase II and III trials. Raymond *et al.* (2011a,b) have reported early in 2011 on their phase III RCT of 37.5 mg sunitinib given continuously vs best supportive care (BSC) in pNET patients who had metastatic low–intermediate (well-differentiated)-grade tumours that had progressed in the last 12 months. Forty-nine per cent of the patients recruited had functional pNETs. Following positive outcome data for patients on sunitinib therapy at the interim analysis, the trial was terminated early having recruited 171 patients (86 receiving sunitinib and 85 receiving placebo). The primary end point of PFS doubled from 5.5 to 11.4 months in the sunitinib group (HR 0.42; CI 0.26–0.66; $P=0.0001$). Disease stabilisation was most often observed with an objective response only seen in 9.3% of cases on sunitinib and 0% of patients receiving placebo ($P=0.007$). It was also reported

that patient quality of life scores were higher and a delay in the onset of symptoms was observed in the treatment group. Generally, patients receiving sunitinib therapy tolerated the treatment well. The most common toxicities were fatigue, diarrhoea, nausea and vomiting and palmar–plantar erythema. Grade 3–4 toxicities when compared to the placebo group included neutropaenia (12 vs 0%), hypertension (10 vs 1%) and palmar–plantar erythema (6 vs 0%). Dose reductions to 25 mg/day were permitted, but mean relative dose intensity was maintained at 91.3%. Following this study, sunitinib is now used in low–intermediate-grade metastatic pNETs that have progressed in the preceding 12 months.

Everolimus and mTOR inhibitors

Everolimus is an inhibitor of the mTOR, a serine threonine kinase that plays a role in key cellular processes including cell growth, signalling and apoptosis. Activation of mTOR occurs in several cancers. It acts as a signal transducer in the PIK3 pathway and is regulated by the tumour suppressor genes *PTEN*, *NF1* and *LKB1* (*STK11*; Cantley 2002, Shaw & Cantley 2006). mTOR is also involved in downstream signalling VEGF and IGF1 signalling pathways (Karar & Maity 2011). Everolimus is approved for use in the treatment of clear cell renal cancer (when sunitinib therapy has failed) and giant cell astrocytoma.

Initial trials of rapamycin and everolimus on neuroendocrine cell lines showed positive results and further preclinical data were obtained using the RIP-Tag2 pNET mouse model (Grozinsky-Glasberg *et al.* 2008a,b, Moreno *et al.* 2008, Chiu *et al.* 2010). In this study, mice were treated with rapamycin (mTOR inhibitor) alone and in combination with the epidermal growth factor receptor (EGFR) inhibitor erlotinib. Monotherapy with rapamycin led to prolonged PFS, whereas the addition of erlotinib increased overall survival (OS). Phase II and III trials of monotherapy with everolimus were already underway when these data were published. Yao *et al.* (2011) reported the outcome of the phase III RCT of everolimus in metastatic low–intermediate-grade pNETs in early 2011 (RADIANT 3 Trial). When compared with the phase II sunitinib study, a very similar improved PFS was observed in the everolimus-treated group (11.4 vs 5.4 months; $P<0.0001$) with no reported improvement of OS to date. Two hundred and seven patients were treated with 10 mg everolimus continuously and 203 received BSC. Patients could crossover to the treatment arm on progression. An objective response

to everolimus was seen in 4.8% patients vs 2.0% in the placebo group – with the majority of patients experiencing disease stabilisation (78% everolimus-treated patients vs 53% on placebo). A subgroup of patients in this study was treated concomitantly with SSAs, the PFS was not statistically significant between everolimus monotherapy and concomitant therapy with SSAs. It was also noted that improved glycaemic control was observed in patients with functioning insulinoma. Everolimus therapy was well tolerated with the most common side effects being stomatitis, rash, diarrhoea, fatigue and upper respiratory tract infections. Grade 3–4 toxicities when compared with the placebo group were stomatitis (7 vs 0%), anaemia (6 vs 0%) and hyperglycaemia (5 vs 2%).

Grade 3–4 toxicities were similar in both RADIANT 2 and 3 trials of everolimus and include stomatitis (7%), diarrhoea (3–6%), fatigue (2–7%), infections (2–5%) and rash (1%). In the event of significant toxicity, dose reduction of everolimus to 5 mg/day, then 5 mg alternate days, was permitted (in RADIANT 3, dose adjustments were required by 59% of patients, and in RADIANT 2, drug-related adverse events led to study discontinuation in 19% of the everolimus group). See Table 2 for an overview of toxicities and clinical monitoring required when treating patients with sunitinib and everolimus.

The UKINETS guidelines for the management of GEP NETs state that sunitinib or everolimus may be used as a line of therapy for patients with advanced (inoperable or metastatic) progressive (radiological evidence of disease progression within 12 months), well-differentiated pNETs (level of evidence 1, grade of recommendation A; Ramage et al. 2012). The more recently published ENETS guidelines recommend that

everolimus may be used after failure of chemotherapy in pNET, and in extrapancreatic NET when other treatment options have failed. Sunitinib is also recommended as second- or third-line therapy for pNET but not for extrapancreatic NET due to the lack of any trials supporting efficacy in this setting (Pavel et al. 2011a,b).

Intestinal NETs

Pavel et al. (2011a,b) have recently published data from the cohort of patients with intestinal NETs (with carcinoid syndrome) recruited to the RADIANT2 trial of everolimus vs placebo. The primary tumour site in this cohort was predominantly of small intestinal origin (51–53% of participants). The majority of patients had low–intermediate-grade (well-differentiated) tumours (77–82%) and 94% were of PS 0–1. Progression-free survival improved from 11.3m with octreotide LAR plus placebo to 16.4m with everolimus and octreotide LAR (HR 0.77, 95% CI 0.59–1.00, $P=0.026$). The most common toxicities in the everolimus + octreotide LAR group when compared with the octreotide LAR monotherapy group were stomatitis (62 vs 14%), rash (37 vs 12%), fatigue (31 vs 23%) and diarrhoea (27 vs 16%). Prespecified subgroup analyses showed consistent benefit in differing primary tumour sites, regardless of previous chemotherapy treatment. Biochemical responses including chromogranin A (CgA) and urinary 5-HIAA were also significant allowing for easily accessible monitoring of response in a clinical setting, toxicity was consistent with previous reports. The trial did not report impact on symptoms or patient-reported outcomes, and overall survival will be impacted by the high rate of crossover from the placebo to treatment arm.

Table 2 Summary of clinical safety monitoring and toxicity assessment for the use of molecularly targeted therapies in patients with neuroendocrine tumours

	Sunitinib	Everolimus
Pretreatment evaluation	Adequate bone marrow, renal and liver function (adequate thyroid function). Blood pressure within normal limits. LVEF > 50%. No ongoing cardiac arrhythmias, or recent thromboembolic disease. Performance status 0–2	Adequate bone marrow, renal and liver function. Adequate lipid concentrations
On treatment frequency of visits	Initial 2 week toxicity check, 4 weekly when stable	Initial 2 week toxicity check, 4 weekly when stable
Expected toxicities	Neutropaenia, diarrhoea, asthenia, erythrodysesthesia, hypertension, thrombocytopaenia	Stomatitis, rash, fatigue, diarrhoea, hyperglycaemia, thrombocytopaenia, infections, pneumonitis, hypercholesterolaemia
On treatment safety monitoring investigations	Clinical examination, vital sign measurement, haematological and biochemistry assessment	Clinical examination, vital sign measurement, haematological and biochemistry assessment. Chest radiograph every 12 weeks

LVEF, left ventricular ejection fraction.

Other pathways that have been targeted in both pancreatic and intestinal NETs

Epidermal growth factor receptor

The EGFR pathway has been previously investigated as a therapeutic target in NETs with limited success. It has been demonstrated that both pulmonary and GEP NETs frequently express EGFR (Shah *et al.* 2006, Rickman *et al.* 2009). Following the observation in the preclinical RIP-Tag2 mouse model that the addition of the EGFR inhibitor erlotinib improved overall survival when used concomitantly with mTOR inhibition (Chiu *et al.* 2010), a phase II clinical trial is currently in progress and due to complete in December 2012 (www.clinicaltrials.gov. NCT00843531 RAD001 and erlotinib in patients with NETs. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT00843531>).

IGF pathway

The IGF system is known to be implicated in the development of several solid tumours including colorectal and breast cancer. Activation of the IGF pathway leads to malignant transformation, increased metastatic potential and activation of VEGF through the HIF response mechanism. GEP NETs are known to express IGFs and their receptors (Wulbrand *et al.* 2000). *In vitro* studies using the BON1 pancreatic lymph node metastasis cell line found that active IGF1 and IGF1R were expressed and that the addition of IGF1 leads to increased excretion of CgA by the cell line (von Wichert *et al.* 2000).

Based on these observations, there are ongoing clinical trials investigating the activity of the IGF1R monoclonal antibody Cetuximab in NETs. See Table 3 for summary.

Novel molecularly targeted therapies under investigation

There are currently 15 phase II and three phase I trials of molecularly targeted therapy in NETs registered with Clinical Trials.gov recruiting globally (reviewed in Table 3; <http://www.clinicaltrials.gov>). The following agents are of particular interest.

Pazopanib

Pazopanib is an orally available angiogenesis inhibitor that targets VEGFR1, -2 and -3; PDGFR α and - β ; and c-kit. It was approved by FDA for the treatment of renal cell carcinoma in 2009. It has a similar side effect and toxicity profile as sunitinib but is reported to be

better tolerated, and it is a desirable novel agent due to its targeting of multiple VEGFRs. Current phase II trials in progress include monotherapy in low–intermediate-grade NETs (www.clinicaltrials.gov. NCT 01099540 Study of pazopanib monotherapy in patients with unresectable, metastatic NET. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT+01099540+>, www.clinicaltrials.gov. NCT 01280201 Pazopanib as single agent in advanced NETs. 2012; Available from: NCT 01280201), both these studies include NETs of pancreatic, intestinal and bronchial origin. Trials of pazopanib in combination with other agents include temozolomide in pNETs (www.clinicaltrials.gov. NCT 01465659 Temozolomide and pazopanib hydrochloride in treating patients with advanced pNETs that cannot be removed by surgery. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT+01465659>) and everolimus during embolisation with SIR spheres (www.clinicaltrials.gov. NCT 01469572 Pasireotide, everolimus and selective internal radioembolisation therapy for unresectable hepatic metastases. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT+01469572>).

Brivanib

Brivanib is a novel agent with dual-inhibitor activity of FGF and VEGF, which shows activity and improves survival in the RIP-Tag2 mouse model of pNETs both first-line and after failure of sorafenib therapy (Allen *et al.* 2011). This agent is planned for further clinical evaluation in early phase trials.

Cabozantinib

Cabozantinib is another novel small molecule kinase inhibitor that inhibits MET and VEGFR2 and has been shown to suppress metastasis, angiogenesis and tumour growth in early phase trials (Yakes *et al.* 2011), an open-label phase II study in advanced pancreatic and GI NETs is due to open shortly (www.clinicaltrials.gov. NCT 01466036 Cabozantinib in advanced pancreatic neuroendocrine and carcinoid tumours. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=01466036>).

Future considerations

It is now widely accepted that both sunitinib and everolimus can be used in selected metastatic pNET patients of low–intermediate-grade who have progressed in the last 12 months. Recent data from the RADIANT 2 trial also suggest that everolimus

Table 3 Review of molecularly targeted therapies under investigation in NETs

Agent	Targets	Phase	Trial details	NCT identifier
Pazopanib	VEGFR1–3, PDGF α and - β , c-kit	II	Monotherapy in low–intermediate-grade pancreatic, intestinal and bronchial NETs	NCT 01099540
	VEGFR1–3, PDGF α and - β , c-kit	I–II	In combination with temozolomide in pancreatic NETs	NCT 01280201
	VEGFR1–3, PDGF α and - β , c-kit	I–II	In combination with everolimus in SIR sphere radio-embolisation	NCT 01469572
Axitinib	VEGFR1–3, PDGF β , c-kit	II	Monotherapy single-arm study in low–intermediate-grade NETs of all types	NCT 01435122
Everolimus	mTOR	II	RAMSETE single-arm trial of everolimus monotherapy in low–intermediate-grade, metastatic, non-syndromic NETs	NCT 00688623
	mTOR	II	In combination with carboplatin/paclitaxel in bronchial NETs/large cell lung cancer	NCT 01317615
	mTOR	II	In combination with erlotinib in low–intermediate-grade pancreatic NETs or paraganglioma (carcinoids excluded from April 2011)	NCT 00843531
	mTOR	Ib	Monotherapy in Chinese patients with advanced pulmonary NET	NCT 01324492
	mTOR	II	Randomised in combination with octreotide +/- bevacizumab in pancreatic NETs	NCT 01229943
	mTOR	I–II	In combination with pasireotide LAR in low–intermediate-grade lung or gastroenteropancreatic metastatic NETs (COOPERATE 1 and 2 studies). Phase II study in pancreatic NETs only	NCT01263353 NCT 01374451
Temsirolimus	mTOR	II	In combination with bevacizumab in low–intermediate-grade pancreatic and intestinal NETs	NCT 01010126
Cixutumumab	IGF1 receptor monoclonal antibody	I	In combination with everolimus and octreotide in low–intermediate-grade NETs	NCT 01204476
Cabozantinib	MET, VEGFR2	II	Monotherapy single-arm study in advanced low–intermediate-grade pancreatic and intestinal NETs	NCT 01466036 (recruiting from 2012)

VEGFR, vascular endothelial growth factor receptor; PDGF, platelet-derived growth factor; c-kit, a receptor tyrosine kinase; SIR, selective internal radiation; mTOR, mammalian target of rapamycin; IGF, insulin like growth factor; MET, a receptor tyrosine kinase.

improves PFS in intestinal NETs; however, this therapy is not approved by FDA at this time. What is unknown is whether these or similar agents are beneficial in other NET histological groups including bronchial NETs and metastatic paraganglioma and whether they would improve clinical outcome in other clinical scenarios such as adjuvant therapy.

Scheduling of molecularly targeted therapy

One of the major challenges associated with the approval of new cancer therapies is to determine their optimal role alongside existing management protocols. This is particularly challenging in rare tumours such as NETs where there is a lack of large-scale RCTs to provide a ‘gold standard’ to work against. Alongside this, in NETs, there are a number of different systemic therapies available often making optimal scheduling difficult to determine.

To date, sunitinib and everolimus have shown benefit in patients with advanced or metastatic disease

with low- and intermediate-grade (well-differentiated) tumours as monotherapy or in combination with SSAs. Historically, streptozocin-based chemotherapy has been beneficial to selected pNET patients with significantly higher RR than those observed with sunitinib and everolimus. It is not known whether treatment with molecularly targeted therapy either before or after chemotherapy improves PFS and quality of life. A European clinical trial is planned to address this question.

It also remains to be determined whether concurrent treatment with targeted therapy and chemotherapy, or of multiple targeted agents, is feasible in terms of toxicity, or warranted in terms of clinical benefit. There is some evidence that combinations of targeted agents lead to unmanageable toxicity, which has in the past led to early termination of trials (Chan *et al.* 2010) (www.clinicaltrials.gov, NCT00947167 A phase II study of pertuzumab and erlotinib for metastatic or unresectable NETs. 2011). There is a phase I/II study

currently recruiting to assess the safety and efficacy of the combination of temozolomide with pazopanib in patients with pNET (www.clinicaltrials.gov. NCT 01465659 Temozolomide and pazopanib hydrochloride in treating patients with advanced pNETs that cannot be removed by surgery. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT+01465659>).

Adjuvant therapy

There is currently no standard adjuvant treatment recommended for NETs following curative surgery (with the exception of goblet cell intestinal tumours), the current 5-year overall survival rate after resection of pNETs is 59.3% (Bilimoria *et al.* 2008).

The use of molecularly targeted treatments in the adjuvant setting is an interesting concept; however at this time, we lack comprehensive global PFS, OS and outcome data, which is required to plan adequately powered adjuvant therapy clinical trials. The collection of this data is necessary in order to identify those patients at highest risk of recurrence who might benefit from this therapy.

Non-pNETs

The role of targeted treatments in patients with non-pNETs is evolving. Preclinical models of bronchial carcinoid cells showed inhibition of growth with everolimus treatment (Zatelli *et al.* 2010). The 44 patients with bronchial NET in the RADIANT 2 trial showed benefit consistent with the general study population: an improvement in PFS from 5.4 to 13.6 months (Pavel *et al.* 2011a,b). This evidence needs to be further validated in a larger patient cohort in order to confirm the role of everolimus in treatment of bronchial NET, which comprises ~30% of the total NET patient population.

The RADIANT 2 trial provided stronger evidence for the use of everolimus in gastrointestinal NET as the majority of patients involved in the study had GI NET and had a clinical significant benefit in PFS of 5.1 months. This is a particularly important finding given that GI NETs are less chemosensitive than pNETs. Carcinoid syndrome is present in <20% of patients with intestinal NETs (Khan & Caplin 2011), and the outcome of the RAMSETE trial looking at the impact of everolimus on non-functioning NETs is awaited.

Sunitinib has been used with some clinical effect in metastatic paraganglioma and pheochromocytoma patients following a series of case reports globally. There is increasing preclinical evidence to suggest the use of angiogenic molecularly targeted therapy in this

tumour type (Joshua *et al.* 2009), and there are two ongoing phase II trials globally investigating the use of sunitinib monotherapy (SNIPP Trial (www.clinicaltrials.gov. NCT 00843037 Study of sunitinib in patients with recurrent paraganglioma/phaeochromocytoma. 2012; Available from: <http://www.clinicaltrials.gov/ct2/results?term=NCT00843037>) and FIRSTMAPP Trial (www.clinicaltrials.gov. NCT 01371201 First international randomized study in malignant progressive pheochromocytoma and paraganglioma (FIRSTMAPP). 2012; Available from: <http://www.clinicaltrials.gov/ct2/show/NCT01371201?term=sunitinib+paraganglioma&rank=2>)).

High-grade tumours

The role of molecularly targeted agents in metastatic poorly differentiated NETs of any origin is unclear. It is usually recommended that these cases are treated with first-line chemotherapy. All the phase III trials to date included well- or moderately differentiated (low- or intermediate-grade) tumours. We know that the underlying genetic and epigenetic changes in these tumours differ depending on grade and this may affect response to targeted treatments. Currently, there is no evidence to support the use of either everolimus or sunitinib in high-grade NETs; however, concomitant therapy with chemotherapy may improve clinical outcome if the combined toxicity is tolerated.

Toxicity management and patient selection

A further challenge in treating patients undergoing these treatments will be managing toxicity, particularly if patients are to remain on treatment for a number of months. The toxicity profiles of both drugs are well documented in the setting of renal cancer, and the adverse events profile noted during the phase III trials in NETs did not demonstrate any unexpected toxicities. However, it remains to be seen whether hypertension can be used as a surrogate biomarker of response (as it is in renal cell cancer) in NET, and whether management of such toxicities requires specific measures in this patient population.

To date, the majority of patients enrolled in the large RCTs have been of good PS (ECOG 0–1). The RADIANT 3 trial included six PS2 patients in each of the treatment and placebo arms (3% enrolled patients) and the sunitinib trial enrolled one PS2 patient in total. Whilst some patients experience severe fatigue on these therapies, others can tolerate treatment with minimal toxicity; therefore in selected PS2 patients, an improvement in PFS and symptomatic control might be achievable with these treatments.

Molecular markers for response to treatment

It is recommended that 'conventional' biomarkers such as chromogranin-A, urinary 5-HIAA and gut hormones where appropriate are used to monitor response to therapy. However, there is a real need to identify and introduce novel biomarkers to aid diagnosis, treatment stratification and predict response to systemic therapy.

Reduced levels of O(6)-methylguanine DNA methyltransferase (MGMT) expression has been associated with response to temozolomide in other solid tumours such as glioma. A study by Kulke *et al.* (2009) examined MGMT expression in pNETs undergoing treatment with temozolomide. This found reduced expression of MGMT in 51% of pNETs (this was not correlated with grade of tumour). When compared with those tumours expressing MGMT, the MGMT-deficient tumours had a significantly higher response rate to temozolomide-based chemotherapy. This immunohistochemistry-based marker shows promise, but at this time requires further validation in larger cohorts.

Circulating tumour cells (CTCs) are emerging as a novel biomarker in NETs, their presence is associated with poorer overall survival and they may have a role in determining response to systemic therapies including chemotherapy, PRRT and molecularly targeted therapy (Khan *et al.* 2011). In prostate cancer, the number of CTCs in patients responding to systemic therapy falls sharply and quickly after commencing therapy; therefore, if the same were to happen in NETs, potentially toxic therapies could be stopped earlier, rather than waiting for 3 monthly interval scanning.

Novel agents

Novel agents targeting the PI3K/AKT/mTOR pathway are in development, and early trials including PI3K inhibitors (Zitzmann *et al.* 2010), IGF1R monoclonal antibodies (Mendivil *et al.* 2011) and second-generation mTORC1/mTORC2 inhibitors are in progress (Jessen *et al.* 2009). These agents show promise but require further validation in the context of large randomised phase III trials. Following a meeting of the NET Task force NCI GI steering committee in 2011, it has been recommended that PFS can be used as a relevant primary endpoint in phase II and III trials as NETs (particularly of low and intermediate grade) often have a long OS and PFS, particularly as the observed radiological RR is low with molecularly targeted therapies (Kulke *et al.* 2011).

Conclusions

The molecularly targeted therapies everolimus and sunitinib have been rationally and successfully applied to the treatment of low–intermediate-grade pNETs. Everolimus was shown to improve progression-free survival by 6.4 months in pNET and 5.1 months in patients with carcinoid syndrome (including gastrointestinal and bronchial NETs). Recently, everolimus has also shown to improve PFS in small intestinal NETs. Sunitinib has demonstrated a similar improvement in PFS of 5.9 months in pNET, novel agents targeted to VEGF as well as mTOR/PI3k/AKT pathway are in development.

With the current evidence available, we propose that sunitinib and everolimus (with SSAs in symptomatic patients) can be used in metastatic low–intermediate-grade pNETs that have progressed in the previous 12 months. It is reasonable to consider changing therapy from sunitinib to everolimus (and vice versa) at progression, although currently there is no clinical trial data to support this. There is increasing evidence supporting the activity of everolimus in intestinal and bronchial NETs; however, there is no FDA approval for the use of everolimus for those patient groups.

What remains to be determined is the exact scheduling of molecularly targeted therapy around other systemic therapies, particularly chemotherapy, clinical trials are planned to address this. Currently, there is no evidence to support the use of adjuvant therapy, and trial outcome data are awaited for the combined use of temozolomide and pazopanib.

In order to optimise patient selection and clinical outcome, identification of predictive and prognostic biomarkers of response for these agents is required. The costs of the systemic therapies discussed in this review vary greatly with PRRT costing up to £25 000 (\$40 000) for a full course of treatment, sunitinib/everolimus costing £2500 (\$4000) per month and chemotherapy costing ~£800 (\$1280) per course. Although the cost of therapy should not determine the choice of treatment, it has implications in countries with public-funded health care systems where restrictions exist in funding certain cancer therapies.

Historically, NET clinical trials did not always distinguish between intestinal NETs from pNETs, as it has only more recently been determined that the biology underlying these two tumour groups is markedly different. This led to inconclusive outcome data from earlier trials. It is now recommended that gastrointestinal, pancreatic and other NETs are treated as separate entities in future clinical trials. Moreover, it has been clearly demonstrated that large-scale phase III

trials are feasible through international collaboration in this rare tumour type and should be continued.

Whilst we are beginning to determine the genetics and pathogenesis of pNETs and utilise molecularly targeted treatment in this disease outside of the research setting, our understanding of gastrointestinal and bronchial NETs requires further development. We await results from large-scale integrated genomic analyses of NET subgroups that will guide the identification of novel clinical biomarkers, relevant signalling pathways and therapeutic targets. With the introduction of the first oral molecularly targeted therapies in pNETs, personalised NET treatment is becoming a reality. Integrated biomarker studies should be incorporated in to clinical trials in order to identify the best biomarkers in response to treatment and overall prognosis. This will prevent our patients receiving therapies that have significant toxicities unnecessarily and guide them towards truly personalised cancer treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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