GEP–NETs UPDATE

Biotherapy for neuroendocrine tumours

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Abstract

Neuroendocrine tumours (NETs) represent a less frequent and heterogeneous group of tumours, which has experienced, in recent years, a significant increase in effective therapeutic possibilities overcoming the disappointing results from chemotherapy. Initial improvements in treatment strategies came from somatostatin analogues (SSAs) that have widely demonstrated a significant improvement in symptomatic relief and tumour control growth by a complex mechanism of action over cell survival, angiogenesis and immunomodulation. Recent investigations have pointed out novel SSAs with a wider binding profile (pasireotide), chimeric molecules against somatostatin receptors and dopamine receptors and the combination with targeted agents, such as mTOR inhibitors or antiangiogenic agents. Immunotherapy is the second cornerstone in NET treatment and has been represented with interferon alpha for a long time, with a demonstrated activity on tumour and clinical response. Its less manageable adverse events have limited its usage. However, different checkpoints in immune system regulation have been effectively targeted in different solid tumours, and novel approaches are currently arising in NETs. In conclusion, biotherapy remains an active treatment strategy for initial approach in patients with NETs. Further investigation on patients' selection, molecular profiles, treatment sequence or combination and optimisation of current and novel biotherapy agents is required.

European Journal of Endocrinology
(2015) **172**, R31–R46

European Journal of Endocrinology

Introduction

Neuroendocrine tumours (NETs) represent a complex, heterogeneous and fairly rare population concerning gastrointestinal (GI), pancreatic, bronchial and thymic tumours, which are the most frequent, and others arising from parathyroid, adrenal, pituitary glands and calcitonin-producing cells of the thyroid. In recent years, the prevalence has increased with an estimated frequency of ~35 cases/100 000 per year (1). However, ~80% of

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www.eje-online.org
DOI: 10.1530/EJE-14-0354

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Table 1 Inhibitory effects of somatostatin receptor subtypes and binding affinities of native somatostatin and synthetic somatostatin analogues (8, 14).

Inhibitory effect	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Hormone secretion					
Growth hormone	+	+			+
Adrenocorticotrophic hormone		+			+
Thyroid-stimulating hormone		+			+
Insulin		+			+
Glucagon					+
Exocrine secretion					
Gastric acid		++			
Amylase					+
Intestinal gastrointestinal secretion	+	+			
Cell proliferation					
Induction of G1 cell cycle arrest	+	+		+	+
Induction of apoptosis		+	+		
Binding affinities (IC_{50} values; nmol/l)					
Somatostatin 14	0.93	0.15	0.56	1.5	0.29
Octreotide	280	0.38	7.1	>1000	6.3
Lanreotide	180	0.54	14	230	17
Pasireotide	9.3	1.0	1.5	>1000	0.7
KE108	2.6±0.4	0.9±0.1	1.5±0.2	1.6±0.1	0.65±0.1

newly diagnosed patients present with metastasis, requiring an effective systemic treatment to prolong survival. The 5-year survival rate for this population is ~40%. Therefore, effective systemic treatment is required (2).

The following review covers two main pillars in current NETs' systemic treatment: somatostatin analogues (SSAs) and immunotherapy (interferon alpha (IFNα)) and newly immune therapies under investigation.

Molecular biology and the rationale behind the use of SSAs and IFN in NETs

Somatostatin is a peptide hormone playing an inhibitory role in exocrine (gastric acid, intestinal fluid or pancreatic enzymes) and endocrine secretion (growth hormone (GH), insulin, glucagon, gastrin, cholecystokinin, vasoactive intestinal peptide and secretin), neurotransmission, immunomodulation and cell proliferation (Table 1). It was initially identified not only in the hypothalamus, but also in the CNS and peripheral nervous system, intestinal tract, endocrine pancreas and immune system. Two different natural active forms of somatostatin are obtained by protein hydrolysis of the pro-hormone: somatostatin 14 and somatostatin 28 (3) (Fig. 1).

A family of five different G protein-coupled somatostatin receptors (SSTRs) have been currently defined (SSTR1–SSTR5). They share approximately half of the amino acid sequence, but with different tumour tissue expression levels and biological functions. SSTR2 is the most commonly expressed SSTR in (GI-NETs) (90%) and pancreatic NETs (80%) (p-NETs) (4). It is also the main SSTR identified in other NETs such as pituitary adenomas (with considerably different SSTR expression between somatotrophinomas and the other tumour types, such as adrenocorticotrophic hormone (ACTH)-, thyroid-stimulating hormone- or PRL-producing tumours), lung NETs, phaeochromocytomas and paragangliomas and neural tumours such as medulloblastomas, meningiomas and neuroblastomas (3). The other SSTRs are slightly less representative (SSTR1 followed by SSTR5, SSTR3 and,

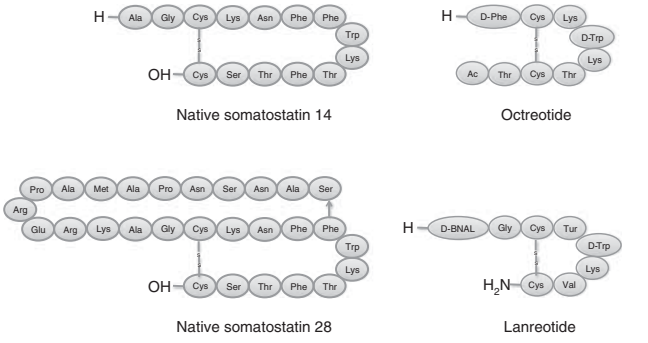

Figure 1
Chemical structure of native somatostatin 14, native somatostatin 28 and synthetic somatostatin analogues (octreotide and lanreotide).

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finally, SSTR4), but their expression is also found in pituitary adenomas, lung NETs, medullary thyroid carcinomas or prostate carcinomas.

Concerning p-NETs, SSTR expression is variable depending on the tumour subtype. Gastrinomas, glucagonomas and VIPomas or parathyroid hormone-related peptide (PTHrP)-related hypercalcaemia usually express SSTR in 80–100% of patients. However, insulinomas, which are subdivided into benign and malignant (5–10%) subtypes, express SSTR in 50–70% of patients. Therefore, SSAs seem to be less effective in symptomatic relief and may worsen hypoglycaemia in those subtypes lacking SSTR expression.

Somatostatins act through direct G protein-coupled receptor activation and indirect ion channel and tyrosine kinase receptors leading to the interruption of cell functions (5). These final results are obtained through a complex signalling cascade due to the differences between receptor subtypes, cells involved, tumour subtypes and the specificity between each SSTR and the molecular pathway activated.

In response to ligand signalling, SSTR1 activates the MAPK pathway, SSTR2 increases SHP1 and epidermal growth factor receptor (EGFR) activity and decreases MAPK activation up-regulating *p21* and *Rb* leading to cell cycle arrest, SSTR3 promotes phosphotyrosine phosphatase (PTP)-dependent apoptosis through *p53* and Bax activity and inhibits vascular endothelial growth factor receptor (VEGFR) and SSTR5 activates PTPs (including SHP1, SHP2 and PTPγ). Overall, cell cycle and proliferation are interrupted, as well as hormone secretion. By contrast, SSTR4 up-regulates the MAPK/ERK1/2 pathway leading to proliferative activity. SSTRs are also related to multiple secondary effectors such as adenylyl cyclase and protein kinase A (6) and ion channel regulation such as K^+ channel, voltage-dependent Ca^{2+} channel, Na^+/H^+ exchanger or AMPA/Kainate glutamate channels (7) (Fig. 2).

Once the signalling cascade is initiated, receptor turnover also has a different behaviour depending on the SSTR subtype: SSTR2 is rapidly recycled, SSTR3 is internalised and degraded through the ubiquitin-dependent pathway and SSTR4 does become internalised (8).

The antiproliferative effect of SSAs is mediated by direct and indirect mechanisms:

Direct effects require SSTR expression by the tumour cells (cell cycle arrest, inhibition of growth factor effect and pro-apoptotic effect):

i) activation of SHP1, SHP2 and r-PTPeta leading to cell cycle arrest by inducing p27kip1.
ii) Inhibition of proliferation by regulation of tyrosine kinase, PTP, nitric oxide (NO) synthase, cyclic guanosine 3′,5′-cyclic monophosphate-dependent protein kinase and RAS signalling.
iii) Induction of apoptosis by up-regulating *p53* and *BAX* through SSTR3 and also *TRAIL (TNFSF10)*, TNFα receptor, dopamine receptor 4 *(DR4 (TNFRSF10A))* and *TNFRI (TNFRSF1A)* through SSTR2, which also down-regulates the anti-apoptotic mitochondrial bcl2.
iv) SSTR1, SSTR3 and SSTR4 activation may inhibit NHE1 channel modifying intracellular pH by realising H^+ and reducing cell proliferation.
v) Overexpression of endogenous connexins (CX26 (GJB2) and CX43 (GJA1)) to construct functional gap junctions.

Indirect antiproliferative effects do not require the tumour to express SSTR (8, 9):

i) Inhibition of growth factor and trophic hormone release (GH, insulin-like growth factor 1 (IGF1), EGF, insulin, glucagon, gastrin, prolactin, cholecystokinin, vasoactive intestinal peptides and serotonin) by calcium depletion. IGF1 is an important key factor in tumour growth, hence its inhibition by central and peripheral mechanisms, such as SSTR2 and SSTR5 activity or through the inhibition of the *IGF1* gene transcription by STAT5b down-regulation mediated by SSTR2 or SSTR3, may reduce tumour proliferation.
ii) Inhibition of angiogenesis, which is involved in tumour growth and metastasis, is mainly due to SSTR2 expression at endothelial cell surface during the angiogenic switch (10). Antiangiogenic effects are achieved by the MAPK/ERK1/2 pathway and endothelial NO synthase down-regulation through SSTR1 and SSTR3 activation and by the inhibition of soluble endothelial growth factors (VEGF, platelet-derived growth factor (PDGF), IGF1 and fibroblast growth factor), through SSTR1–SSTR3 and SSTR5.
iii) Immunomodulatory effect. SSTRs are also present in immune cells and are able to mediate immune and inflammatory reactions through IFNγ (IFNG), TNFα (TNF) and IL1β (IL1B) release. SSAs also inhibit natural killer cell activity and lymphocyte proliferation.

IFNα has been developed in NETs based on its effective activity on cell proliferation and differentiation. It enhances the cytoplasmic messengers JAK1 and TYK2 to stimulate the STAT (1, 2, 3 and 5) transcription factor

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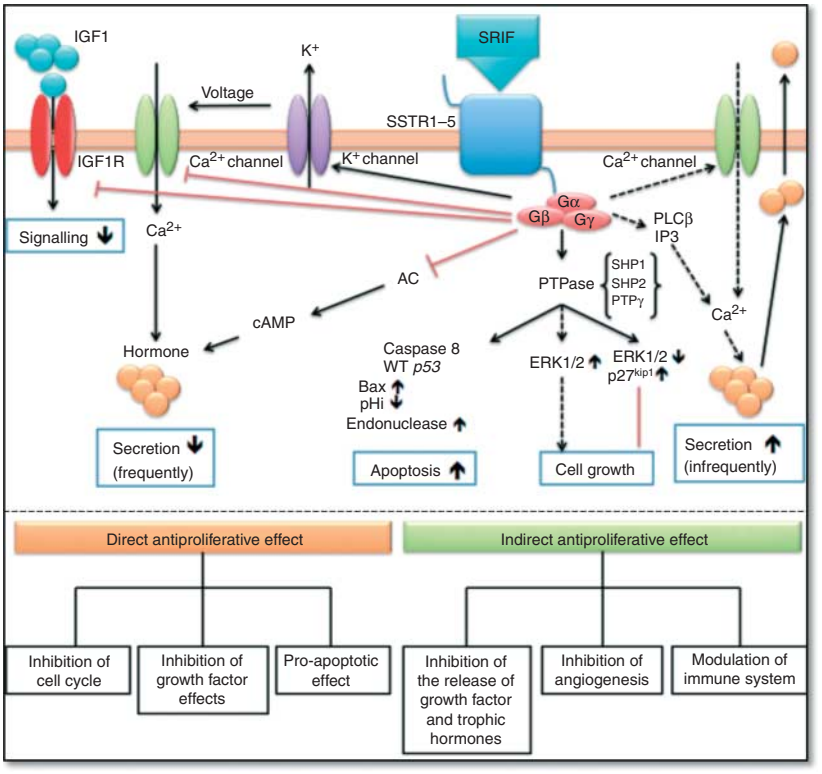

Figure 2

Direct and indirect mechanisms of action mediated by somatotrophin release-inhibiting factor (SRIF) receptor leading to changes in hormone secretion, apoptosis, cell growth, angiogenesis and immunomodulation.

that creates the ISGF3 complex with the IFN regulatory factor 9 (IRF9; p48) (11). In addition, IFN (IFNA) induces apoptosis by activating proteolytic enzymes.

The anti-angiogenic effect is mediated by decreasing the *VEGF* (*VEGFA*) mRNA expression, which is inhibited by reducing the transactivation activity of the transcription factors Sp1 and Sp3, but not interfering with the ability to bind DNA. IFN also reduces the microvessel density as observed in decreased CD31 immunostaining in tissue samples (12).

Other functions such as the induction of tumour mesenchyme and immunomodulation by activation of T lymphocytes are also associated with IFN treatment. IFN is able to induce a cell cycle arrest in S-phase and interrupts the progression to G2/M cell cycle phase by interfering with cyclin-dependent kinase function (inhibiting cdk2 function and cyclin B activation) and upregulating the kinase inhibitors *p21* and p27 (13).

The expression of suppressor of cytokine signalling proteins has been proposed as a resistance mechanism to the antiproliferative activity of IFN by interfering with JAK/STAT downstream cascade.

SSAs and IFNα in the past

Somatostatin analogues

Based on the pre-existing strong rationale, different SSAs have been developed in the management of NETs. The natural role of somatostatin lasts only for a short period of time due to its short half-life of 2–3 min because of rapid enzyme degradation or inactivation through the cleavage site by restriction enzymes, requiring continuous i.v. infusion to maintain the activity. This limitation was overcome with the new synthetic SSAs that are more stable and, consequently, more active against tumour growth. However, even if natural somatostatin has a similar affinity to all SSTRs, synthetic derivatives have a restricted receptor affinity preference. Octreotide and lanreotide bind to SSTR2 and SSTR5 with the highest affinity,

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followed by SSTR3. However, a low affinity has been reported for SSTR1 and SSTR4 binding. A new somatostatin compound, pasireotide (SOM230), has demonstrated a high affinity for SSTR1–SSTR3 and SSTR5 (5), and KE108 has shown a high affinity for all SSTRs. The different binding affinities are described in Table 1.

The carcinoid syndrome that appears in the context of serotonin, substance P, tachykinins and/or other peptide overproduction represents a cornerstone in NET treatment due to the interference in patients' quality of life. Main clinical features are flushing, diarrhoea and abdominal pain, but severe cases may include bronchospasm, tachycardia, hypo/hypertension or fibrous changes in the endocardium and the right heart valves (14). SSAs represent the first biotherapeutic agents able to improve this dramatic situation for patients and, indeed, achieve antitumour response and outcome benefit. In fact, SSAs are able to achieve symptomatic relief and a reduction in tumour markers (urinary 5 hydroxyindoleacetic acid (u5HIAA) or chromogranin A (CgA)) in 30–70% of patients. Tumour regression occurs in $<10\%$ of patients, but stable disease (SD) occurs in $\sim 50\%$ of patients.

Octreotide was the first SSA available for medical use and was approved by the Food and Drug Administration (FDA) in 1987 for symptom control in functioning NETs (carcinoid syndrome, glucagonoma or Verner–Morrison syndrome). Later on, for more than 20 years, octreotide has been reported to be clinically effective over other p-NETs (VIPoma and gastrinomas or insulinomas), ectopic ACTH secretion with Cushing's syndrome, ectopic GH-releasing hormone secretion, and oncogenic osteomalacia and hypercalcaemia caused by ectopic PTHrP secretion. An additional antiproliferative activity from SSAs has been demonstrated by the experience on cultures derived from medullary thyroid carcinoma cell lines showing an inhibitory effect on cell proliferation and, in GH-producing pituitary adenoma cell lines, also showing an inhibitory effect on hormone release (15) and in animal models. Thereafter, in case series reports (16) and recently in clinical trials, positive results have been identified. The highest affinity is against SSTR2, followed by SSTR5 and SSTR3. The substitution of three amino acids prevents enzyme degradation, increasing the half-life from 2–3 to 90–120 min with a pharmacodynamic activity between 8 and 12 h. Octreotide was initially provided as s.c. immediate-release injection, but the following long-acting repeatable formulation encapsulated in microspheres of poly-DL-lactide-co-glycolide-glucose (octreotide LAR) was demonstrated to be as effective as the short-acting formulation with the main advantage of a lower number of injections favouring patients' compliance (17). The octreotide LAR pharmacokinetics allows its administration every 28 days and leaves the short-acting octreotide as a salvage therapy for breakthrough symptoms.

From retrospective trials reporting the meaningful benefit of octreotide in symptom relief, initial observations pointed out the additional ability to tumour shrinkage and further trials were developed (Table 2). A prospective German phase II trial assessed the efficacy of octreotide three times a day at increasing doses in 52 patients with previously confirmed disease progression (18). Nineteen patients (36%) presented SD with a median duration of response of 18 months. A trend to a better response was identified in the subgroup of patients with intestinal tumour origin and carcinoid syndrome. Treatment with high-dose octreotide 500 μg three times a day did not seem to offer additional benefit in tumour growth control. Those results were consistent with a contemporary phase II study that included 58 patients receiving 500–1000 μg three times a day (19), but slightly lower than those from Saltz *et al.*, which investigated the activity of immediate-release octreotide in 34 patients with gastroenteropancreatic (GEP)–NETs (20). No objective response was identified, but 50% (17/34) of patients showed SD for at least 8 weeks and 71% of patients with symptomatic disease had symptom relief and hormone level reduction with a median survival of 22 months. The low rate of tumour shrinkage has been associated with the intermediate affinity to SSTR3, which is involved in apoptosis and tumour growth control.

Those initial results, although promising, come from trials characterised by a low sample size, heterogeneous baseline patients' features with different tumour locations, previous treatment lines, functional status, proliferation index, SSTR expression, histological subtypes or confirmed disease progression at study enrolment that may lead to a selection bias in some patients. A prospective single-centre study including a homogeneous group of 21 patients previously untreated with non-functioning unresectable p-NETs investigated the role of octreotide LAR 20 mg every 28 days. Clinical outcomes showed that the median survival was 45 months with a 5-year survival rate of 52.4% and a 5-year progression free survival (PFS) rate of 32.1%. Long-term SD was achieved in 38% (8/21) of patients. In addition, a correlation among Ki67 $\geq 5\%$, weight loss, lack of abdominal pain and CgA increment during follow-up with poor prognosis was obtained (21). The analysis of predictive biomarkers was also investigated in a retrospective trial with 43 patients with stage III and IV p-NETs treated with octreotide LAR (22). There was a benefit in the duration of

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Table 2 Development of somatostatin analogues (SSAs) in neuroendocrine tumours (NETs).

	Study design (*n*)	Status of remission at start	Tumour type	Grade	Functionality	Biochemical response	Radiological response (PR+SD)	Symptomatic response	Survival (months)
Octreotide									
Arnold (4)	II (103)	PD (52)	Carcinoid Pancreatic	–	31 (60%)	29%	0+19/52 (36%)	45%	–
Bartolomeo (6)	II (58)	PD	Carcinoid Merkel cell MTC Pancreatic	–	15 (25%)	10/15 (77%)	2/58 (3%)+27 (47%)	6/15 (40%)	22 months (carcinoids NR)
Saltz (8)	II (34)	PD	Carcinoid Pancreatic UK origin	–	21 (62%)	7/21 (33.3%)	0+17 (50%)	15/21 (71%)	NR
Butturini (9)	IV (21)	–	Pancreatic	WD	0	6/13 (46%)	0+8 (38%)	8/8 (100%)	45 months
Jann (11)	RT (43)	SD=5 PD=23	Pancreatic	WD+MD	19 (44%)	NA	3 (6.6%)+25 (58%)	NA	98 months
Rinke (49)	III (85)	–	Midgut UK origin	WD	33 (39%)	9/26 (O) vs 4/30 (P)	1+28/42 (O) vs 1+16/43 (P)	9/17 (O) vs 4/19 (P)	NR TTP: 14.3 vs 6.0 months
Lanreotide									
Eriksson (15)	II (19)	–	Carcinoid Pancreatic	–	2/6	7/13 (54%)[a] 8/18 (44%)[b]	1 (5%)+12 (63%)	$P<0.05$	–
Wymenga (17)	II (55)	PD	Carcinoid Pancreatic	–	31 (56%)	9/33 (27%)[a] 9/27 (33%)[b]	2/31 (6%)+25/31 (81%)	11/29 (38%)	–
Ducreux (19)	II (46)	PD–3 months	GEP Lung	–	30 (65%)	15/37 (40%)[a] 13/36 (36%)[c]	2/39 (5%)+19/39 (49%)	12/30 (40%)	–
Bajetta (20)	III (60)	PD if previously treated	GEP Lung	WD	19 (32%)	4/56 (14%) (MP) vs 8/56 (30%) (ATG)	1+18 (MP) vs 0+19 (ATG)	2/2 (MP)+9/9 (ATG) (100%)	PFS, $P=0.8857$
Martin-Richard (22)	II (30)	PD–6 months	GEP Lung	WD	19 (63%)	21/30 (70%)	1/30 (4%)+24/30 (89%)	8/9 (89%)	PFS 12.9 months
Caplin (51)	III (204)	SD=196	Midgut Hindgut Pancreas UK	WD+MD	0	OR 15.2 ($P<0.0001$)	–	–	NR PFS: NR vs 18 months
Pasireotide									
Kvols (57)	II (45)	PD not mandatory	GEP Lung	–	45	Precise decrease NA	13/23 (57%)	12/44 (27%)	–

n, Number of patients included; RT, retrospective; PR, partial response; SD, stable disease; PD, progressive disease; MTC, medullary thyroid carcinoma; GEP, gastroenteropancreatic; UK, unknown; NA, not available; NR, not reached; WD, well differentiated; MD, moderately differentiated; O, octreotide group; P, placebo group; PFS, progression free survival; MP, lanreotide microparticles; ATG, lanreotide autogel.
[a]u5HIAA.
[b]CgA.
[c]Serotonin.

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response for patients with Ki67 of <5% (15 months, $P=0.009$; $n=12$) vs Ki67 of 5–10% (12 months, $P=0.036$; $n=20$) vs Ki67 of >10% (4 months; $n=7$).

Octreotide was usually well tolerated with rare treatment interruptions due to adverse events. The most frequent side effects occurring in 10–20% of patients were local pain at the site of injection and GI disturbances that usually resolved during treatment. By contrast, cholelithiasis may appear with prolonged SSA administration in ~20% of patients, but usually is asymptomatic.

Lanreotide has been introduced as an SSA with a similar biochemical structure, affinity profile to SSTR and activity as octreotide (23) (Table 2). An open pilot phase II trial investigated the role of immediate-release lanreotide at increasing doses in 19 patients, with previously treated functioning metastatic GEP–NETs (24). A partial response (PR) >50% was obtained in one patient and SD in 12 patients with a biochemical response rate (u5HIAA and CgA) of 58%. The introduction of a prolonged release formulation was developed for its administration every 2 weeks. A phase II trial was conducted in 55 patients treated with lanreotide LAR 30 mg and clinical response was identified in 42.1 and 47.6% of patients with diarrhoea or flushing as the main symptoms respectively (25). Disease control was achieved in 87% of patients with a mean duration on treatment of 20.7 weeks. Most adverse events were mild GI events in 26 out of 53 patients (abdominal pain, meteorism and cholelithiasis). Another prospective phase II trial, which strengthen the antiproliferative role of lanreotide, evaluated it at a dose of 30 mg every 10–14 days in 46 patients with carcinoid syndrome and 16 patients without symptomatic GEP–NETs (26). From evaluable patients, a disease control rate was identified in 66.6 and 50% of patients with and without tumour-related symptoms respectively. Symptom relief was completely achieved in 40% of patients within the first month of treatment. The activity of the long-acting lanreotide autogel 120 mg every 6 weeks has been compared with lanreotide microparticles 60 mg every 3 weeks in an Italian non-inferiority phase III trial (27). Results demonstrated a comparable efficacy in tumour growth control (67% in both groups) and biomarkers (57.2% in the microparticle group and 59.2% in the autogel group) with both therapies. Furthermore, a prospective single-arm phase II trial (28) also assessed the activity of long-acting lanreotide autogel at a dose of 120 mg every 28 days. Median PFS was 12.9 months and response to treatment showed a disease control rate of 93%. Predictive factors were also investigated with lanreotide in a retrospective review including 68 patients with well-differentiated GEP–NETs (29). The results from the multivariate analysis showed that Ki67 ≤5% (HR 0.262; $P=0.009$), pretreatment stability (HR 0.241; $P=0.008$) and hepatic tumour involvement ≤25% (HR 0.237; $P=0.004$) were significantly associated with tumour growth control with lanreotide.

There are several mechanisms of resistance to SSAs, which have been proposed in the last years. First, the phenomenon called tachyphylaxis, desensitisation or downregulation in the number of SSTRs in cell surface. It is due to the proliferation of cells with SSTR2 deficiency, the induction of functional changes involving different expression patterns of SSTRs at the cell surface, the increase in SSTR subtype expression that SSAs do not bind with a high affinity (8) or the SSTR2 internalisation and downregulation after a prolonged exposure to an agonist. Secondly, the development of functioning mutation forms of SSTRs (such as SSTR5/MD4 in human pituitary tumours) (30). Thirdly, the generation of antibodies against SSAs. Fourthly, the modification in regulatory proteins (amphiphysin IIb) that are involved in SSTR stabilisation and degradation. Controversial data are available about the median time of the development of these resistance mechanisms. Different strategies have been proposed to overcome the resistance to SSAs, such as the administration of high-dose treatment (octreotide/lanreotide), the introduction of targeted agents to multiple SSTRs or the development of chimeric SSTR/DR molecules.

The efficacy in sequential administration of SSAs was investigated in 15 patients progressing to lanreotide LAR 30 mg every 14 days for a median time of 8 months and receiving octreotide at a dose of 20 mg every 28 days as second-line treatment. Radiological tumour response (PR, 7% ($n=1/15$) and SD, 40% ($n=6/15$)) and clinical benefit (82% ($n=12/15$)) were maintained with the sequential administration of SSAs (31). The lack of cross-resistance between octreotide and lanreotide may be due to different pharmacokinetic or pharmacodynamic profiles, distinct receptor-binding affinity and the development of tumour tolerance or desensitisation mechanisms (32). After the administration of a single dose of long-acting octreotide, there is a rapid concentration increase within the first day followed by a decrease on days 2–6 and a new increment from days 8 to 14 achieving a plateau level that is dose dependent and lasts until day 42 (33). By contrast, lanreotide LAR experiences a peak concentration within the first 24–48 h followed by a steady decrease during the following period. Besides, a study with 40 healthy volunteers demonstrated that the octreotide

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pharmacokinetic profile was more stable and predictable with a more stringent dose-proportional relationship, suggesting a potential greater benefit from an increasing treatment dose (32). However, a superior dose has not been approved at the moment. Concerning lanreotide, some previous studies also identified a benefit from up-titrating patients with lanreotide LAR or immediate release (15) showing comparable efficacy and safety profiles.

Interferon alpha

IFNα has been investigated in non-randomised trials demonstrating antitumour effect by the time the antiproliferative activity of octreotide was under debate (Table 3). However, the potential severe adverse events limited its prescription. Öberg *et al.* (34) initiated the development of IFNα in carcinoid tumours with a pilot study published in 1983 and updated in 1986, demonstrating promising results in tumour growth control. A subsequent retrospective review including 111 patients with liver metastatic carcinoid tumours (62% were previously treated) showed a disease control rate of 54% with a median duration of response of 32 months (35). The survival results were compared with a control group treated only with chemotherapy and overall survival (OS) was 8 months ($n=19$) compared with 64 months in the group receiving IFNα after chemotherapy and 80 months in the group receiving IFNα as first-line treatment. The main adverse events were, as expected, flu-like syndrome, fatigue, weight loss and myelotoxicity. Overall, a comprehensive review on the role of IFNα in carcinoid tumours showed a median biochemical response rate of 63% and radiological overall response rate (ORR) of 20% with IFNα at a dose of 3–9 MU three or seven times per week (36). In p-NETs, the efficacy was demonstrated after failure to first-line chemotherapy showing a biochemical response of 63% (20/32) with a median duration of response of 20.5 months (37). The adverse events and the high-dose strategies for greater responses compromise the long-term treatment with IFNα, even in responders. Therefore, to overcome this concern with human leukocyte IFN, investigators developed recombinant IFNα2a. However, to achieve clinical efficacy, doses needed to be higher. An Italian trial conducted with 53 patients previously treated or treatment naïve ($n=16$) received recombinant IFNα2a at a dose of 6 MU daily for 8 weeks followed by three times per week (38). Efficacy assessment at 6 months showed a disease control rate in 42.6% of patients. In functioning tumours, symptom relief was observed in nine patients (64%) and biochemical response in eight patients (53%). Response to treatment was observed within 2 months. However, a report from Öberg *et al.* (39) pointed out the

Table 3 The role of IFN in clinical trials.

	Study design/ treatment	Number of patients	Status of remission at start	Tumour type	Function-ality	Biochemical response	Radiological response (PR+SD)	Symptomatic response
Öberg (34)	II/IFNα	36	PD	Carcinoid	–	16/36 (44.4%)	6	17/36 (47%)
Öberg (39)	Retrospective/ IFNα2b	20	–	Carcinoid	–	10/20 (50%)	2/17 (12%)+ 14/17 (82%)	NA
Eriksson (37)	II/IFNα	84	Post-chemo-therapy PD	Pancreatic (25 benign/59 malignant)	45/59 (76%)	18/32 (53%)	7/32 (22%)+ 5/32 (16%)	NA
Bajetta (38)	II/rIFNα2a	53	PD	Carcinoid Pancreatic Lung Merkel cell MTC Breast	14/53 (26%)	8/15 (53%)	5/49 (10%)+ 16/49 (33%)	9/14 (64%)
Pavel (40)	Retrospective/ PEG–IFNα2b	17	PD	Carcinoid Pancreatic UK	11/17 (65%)	6/15 (40%)	2/17 (12%)+ 11/17 (65%)	8/11 (70%)
Öberg (35)	Retrospective/ STZ+5FU	19	–	Carcinoid	–	47/111 (42%)	16/111 (15%)/ 43/111 (39%)	76/111 (68%)
	CT→IFNα	68						
	IFNα	43						

PR, partial response; SD, stable disease; PD, progressive disease; MTC, medullary thyroid carcinoma; UK, unknown; NA, not available.

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development of neutralising IFN antibodies during treatment with IFNα2a. Results suggested that physicians should be aware of this phenomenon in patients who suddenly become unresponsive to treatment and it may be solved by the substitution to human leukocyte IFN. In 2006, results from a cohort of 17 patients with well-differentiated GEP–NETs demonstrated a good tolerability and efficacy from PEG–IFNα2b in patients with tumour progression secondary to IFNα interruption due to toxicity (40). In the absence of randomised trials, this treatment strategy was suggested as an alternative in patients who do not tolerate the previous treatment with IFNα.

The role of IFNα in combination with chemotherapy in high-grade NETs has been initially studied in 25 patients with rapidly progressive tumours in combination with a continuous fluorouracil infusion (41). Acceptable results were obtained with an ORR of 41.6% and a median duration of response of 20 months; but tolerance was disappointing in this and following trials to justify further investigation at that time. However, currently, the addition of IFN to fluorouracil is being assessed in some European centres in the adjuvant setting of Globet cell carcinoid of the appendix, due to its intermediate behaviour between carcinoid tumour and adenocarcinoma.

SSAs and IFNα in combination

The addition of IFNα to SSAs has been investigated in several trials in order to optimise the response in tumour growth, overcome the resistance to SSAs and extend the duration of response (42). Despite earlier non-randomised studies that showed promising results from the combination therapy, the last randomised trials presented controversial results for the whole group of patients, questioning the real benefit from the combination over each treatment administered alone (Table 4) (43, 44). Authors wondered whether the sequence would be a better approach rather than the combination therapy. On the one hand, there are limitations from the initial retrospective single-centre trials, such as the inclusion of heterogeneous cohort of patients in terms of previous treatments, tumour load, and primary location site and the different SSA or IFNα dose administration due to individual titration. On the other hand, limits from the randomised trial were the early trial termination due to slow

Table 4 Trials investigating the combination of somatostatin analogues (SSAs) and interferon (IFN).

	Study	*n*	Location primary	Baseline characteristics	Functional tumours	Treatment	Disease control rate	Survival
Janson (42)	Nonrandomised Prospective	55 (18[a])	Foregut (1) Midgut (43) Unknown (11)	Prior IFNα (37)	51/55	Octreotide 100 μg bid+2–6 MIU IFNα tid	86% (94%[a])	–
Frank (63)	Open prospective	21	Pancreas (8) GI (7) Unknown (6)	WD Prior octreotide (16)		Octreotide 200 μg tid+5 MIU IFNα tiw	1CR/13 (67%)	68 vs 23 months[b]
Faiss (43)	Randomised Prospective Three arms	80	Foregut (36) Midgut (30) Hindgut (3) Unknown (11)	WD Treatment naïve	29/80	Lanreotide 1 mg tid (L) ±5 MIU IFNα tiw (IFNα)	8/25 (L)+8/27 (IFNα)+ 7/28 (L+ IFNα)= 28.7%	PFS 1 year rate: 44 vs 44.4 vs 50%
Kölby (64)	Randomised Prospective Two arms	68	Midgut (68)	WD	68/68	Octreotide 100 μg bid–200 μg tid±3–5 MIU IFNα 5/w	–	OS 5 years rate: 36.6 vs 57% (P=0.132) PFS: HR 0.28, P=0.008
Arnold (44)	Randomised Prospective Two arms	109	Pancreas (38) Duodenum (2) Midgut (45) Unknown (20)	WD Prior octreotide (7+6)	42/109	Octreotide 200 μg tid±4.5 MIU IFNα tiw	27 (50%) vs 23 (45%) (at 3 months)	OS: 51 vs 35 months (P=0.55)

GI, gastrointestinal; WD, well-differentiated; OS, overall survival; PFS, progression free survival.
[a]Nineteen patients resistant to octreotide dose escalation added treatment with IFN.
[b]Responders vs non responders.

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Table 5 Trials in progress combining targeted agents with SSAs.

Treatment	Study design	Inclusion criteria	Primary endpoint	Clinical trial number
Axitinib (+octreotide)	Phase II	NET (non pancreas origin)	PFS	NCT01744249
Bevacizumab+pertuzumab (+octreotide)	Phase II	NET	ORR	NCT01121939
Cixutumumab (+octreotide)	Phase II	NET	PFS	NCT00781911
Cixutumumab+everolimus (+octreotide)	Phase I	NET	DLT Safety PD	NCT01204476
Everolimus+bevacizumab	Phase II	p-NET	PFS	NCT01229943
Everolimus (+/−pasireotide)	Phase II	p-NET	PFS	NCT01374451
IFNα2b vs bevacizumab (+octreotide)	Phase III	Carcinoid	PFS	NCT00569127

DLT, dose-limiting toxicities; PD, pharmacodynamic markers.

accrual, the heterogeneous population, the unblinded design or the heterogeneity in radiological tumour assessment that prevents from definitive conclusions about the advantage of a combination therapy (Table 5).

SSAs and targeted agents in combination

NETs are highly vascularised tumours that have demonstrated overexpression of VEGF, VEGFR (VEGFR1 (FLT1)/VEGFR2 (KDR)) and PDGF receptor (PDGFR (PDGFRB)), increasing the interest to inhibit the angiogenesis pathways as a treatment strategy. Furthermore, the overactivation of the serine–threonine kinase mTOR, that enhances cell proliferation, cell growth, metabolism and angiogenesis, is also a key target to inhibit in NETs.

The RADIANT 2 phase III trial (45) included patients with low and intermediate NETs randomised to everolimus 10 mg/day plus octreotide LAR 30 mg every 28 days or placebo plus octreotide at the same doses. Median PFS was 16.4 months for the experimental group compared with 11.3 months for the placebo plus octreotide group (HR 0.77, 95% CI 0.59–1.0; $P=0.026$). Investigation of this combination is ongoing with other targeted agents, such as antiangiogenic drugs like bevacizumab in combination with octreotide (46). The phase III trial by Raymond *et al.* (47) demonstrated the benefit of sunitinib compared with placebo in patients with well-differentiated p-NETs (PFS 11.4 vs 5.5 months respectively; HR 0.418, $P<0.001$) (47). Sixty-eight (40%) patients received SSAs in combination with sunitinib and the benefit of the TKI was identified in both groups of treatment. In addition, retrospective results from a Spanish cohort treated with sunitinib ($n=61$) or everolimus ($n=73$) combined with lanreotide autogel showed interesting results in estimated PFS at 6 and 12 months (79.5 vs 89.3% and 68.6 vs 73% respectively) (48). Pazopanib has also been studied in the phase II trial that firstly introduced the concept of sequential therapy with targeted agents in NETs. Patients were refractory to at least other antiangiogenic and/or mTOR inhibitors and were allowed to receive concomitant SSAs. Results, although in a small number of patients, show a trend towards better outcome with the combination of pazopanib plus SSA (PFS=12.4 months) compared with pazopanib (PFS=6.8 months) (30). This pharmacological combination, with a strong rationale based on different mechanisms of action, possible synergistic effects and promising initial results, is undergoing further investigation.

Current role of SSAs

In 2009, Rinke *et al.* (49) published the first randomised, placebo-controlled trial with octreotide LAR 30 mg in well-differentiated midgut NETs in order to determine the real benefit on tumour growth control and outcome from SSAs (Table 6). The patients' enrolment stopped earlier than planned (85 patients included and 67 patients with confirmed disease progression) after the interim analysis results. The study demonstrated a significantly different time to tumour progression explained by the antiproliferative benefit from octreotide; 14.3 months (95% CI 11.0–28.8 months) for the octreotide group vs 6.0 months (95% CI 3.7–9.4 months) for the placebo group (HR 0.34; 95% CI 0.20–0.59, $P=0.000072$). Patients with or without carcinoid syndrome showed a similar treatment response (14.3 vs 5.5 months and 28.8 vs 5.9 months respectively). Greater hepatic tumour burden (more than 10%) was suggested as a negative prognostic factor in the per-protocol subgroup analysis (HR 2.63, $P=0.0023$). OS was not reached (NR) in the treatment

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Table 6 PROMID and CLARINET clinical trials (49, 50).

Clinical trial	PROMID[a]	CLARINET
Study design	Randomised, double-blind, placebo-controlled, phase III trial	Randomised, double-blind, placebo-controlled, phase III trial
Number of patients	85	204
Treatment	Octreotide LAR 30 mg (42 patients) vs placebo (43 patients)	Lanreotide autogel 120 mg (101 patients) vs placebo (103 patients)
Tumour stage	Locally unresectable/metastatic	Locally unresectable/metastatic
Tumour location	Midgut: 64 (75.3%) Unknown origin: 21 (24.7%)	Midgut: 73 (35.7%) Hindgut: 14 (6.8%) Pancreas: 91 (44.6%) Unknown/others: 26 (12.9%)
Ki67	≤2% in 81 (95%) patients (not pre-specified)	<10%
Grade of differentiation	Well differentiated (G1)	Well/moderately differentiated (G1/G2)
Tumour-related symptoms	Symptomatic (39%)/asymptomatic (61%)	Asymptomatic
Previous treatment lines	Treatment naïve: Exclusion criteria: previously treated with SSAs for ≥4 weeks, or previous treatment with IFNα, chemotherapy or chemoembolisation	Previous treatment allowed: Exclusion criteria: previously treated with SSAs, IFNα, chemotherapy or chemoembolisation ≤6 weeks previous to randomisation 84% of patients were treatment naïve
Surgery primary tumour	56 (66%)	79 (38.7%)
Liver metastasis	73 (85.8%) <10% tumour load: 52 (61.2%) >10% tumour load: 21 (24.6%)	204 (100%) ≤25% tumour load: 137 (67.1%) >25% tumour load: 67 (32.8%)
Primary endpoint	Time to tumour progression (TTP)	Progression-free survival (PFS)
Progressive disease	Unknown	Stable disease in 96% of patients
Efficacy endpoints	SD–6 months: 67% in octreotide group (O) vs 37.2% in placebo group (P) ($P=0.0079$) TTP: 14.3 months (O) vs 6.0 months (P) ($P=0.000072$)	PFS: NR in lanreotide group (L) 18.0 months in placebo group (P) ($P=0.0002$) G2 tumours: NR (L) vs 12.1 (P) months Hepatic tumour load: 24.1 (L) vs 9.4 (P) months p-NET: NR (L) vs 12.1 (P) months
Serious adverse events	11 patients (O) vs ten patients (P)	25 patients (L) vs 32 patients (P)
Treatment discontinuations due to AE	Five patients (O) vs zero patients (P)	Three patients (L) vs zero patients (P)

AE, adverse events; NR, not reached.
[a]Deaths in lanreotide group, 19/101 and deaths in placebo group, 17/103.

group compared with 84 months in the placebo group (HR 0.85; 95% CI 0.46–1.56, $P=0.59$). However, the majority of patients in the placebo arm received octreotide at disease progression interfering in the real survival efficacy of octreotide (50). The greatest benefit was observed in patients with a lower liver tumour involvement (<10%) and resected primary tumour (27.1 vs 7.2 months, $P<0.0001$). A less remarkable benefit from octreotide was observed in patients with higher liver tumour involvement (4.6 vs 2.8 months, HR 0.71). After the results from the PROMID trial, octreotide LAR 20–30 mg was recommended for patients with recurrent or unresectable metastatic carcinoid tumours from any location and irrespective of functional status, symptoms and progression status.

Lanreotide has also been investigated under a phase III trial. Based on the lack of knowledge of the role of SSAs in patients with higher histological grades and greater liver tumour load, a phase III randomised placebo-controlled trial was developed for patients with G1/G2 non-functioning GEP–NETs (51). The CLARINET trial randomised 204 patients to receive lanreotide autogel 120 mg ($n=101$) vs placebo ($n=103$). The primary endpoint of PFS was achieved demonstrating a 53% reduction in disease progression with the SSAs (NR with lanreotide autogel (32 events) and 18 months with placebo (60 events); HR 0.47; 95% CI 0.30–0.73, $P=0.0002$). The subgroup analysis according to primary location site showed that PFS for midgut NETs ($n=73$) was NR vs 21.1 months (HR 0.35; 95% CI 0.16–0.80, $P=0.0091$) and for p-NETs ($n=91$) was NR vs 12.1 months (HR 0.58; 95% CI 0.32–1.04, $P=0.0637$) for lanreotide and placebo respectively. Biochemical response in CgA levels ≥50% from baseline also favoured lanreotide (OR 15.2; 95% CI 4.29–53.87, $P<0.001$). Tolerance to treatment was consistent with previous studies.

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Novel biotherapy agents in NETs

Pasireotide (SOM230)

Instead of creating new compounds with a more specific binding profile, the syntheses of new compounds that mimic the natural somatostatin have been investigated. This is based on the identification of various SSTRs expressed in each GEP–NET (52). For a complete anti-proliferative effect against tumour expressing different SSTR subtypes, it seems that targeted agents with a multi-receptor binding profile should be more effective. Pasireotide (SOM230) is a cyclohexapeptide structure that binds with a high affinity to SSTR1, SSTR3 and SSTR5; with a slightly lower affinity to SSTR2 and no relevant affinity to SSTR4. Its unique structure prevents from proteolytic degradation achieving a half-life of 12 h. The critical amino acids that mediate the universal affinity from natural somatostatin to all five SSTRs are substituted by amino acid analogues that keep this binding property (53). Recent investigations have been able to determine the importance of not only the regions Trp^8 and Lys^9 but also the adjacent regions Lys^4, Phe^6, Phe^7 and Phe^{11} in the universal binding from somatostatin 14 (54). This broad binding profile confers to pasireotide a critical role in tumours refractory to octreotide or lanreotide (55).

Initial preclinical results in cell lines expressing human recombinant SSTRs (52) confirmed the nanomolar or subnanomolar potency of pasireotide over SSTR1–SSTR3 and SSTR5 without an agonist action over SSTR4. Interestingly, pasireotide showed a significantly high affinity to SSTR5, even greater than natural somatostatin 14. Further preclinical studies in animals (53) demonstrated that the multiligand SSTR binding confers some advantages by the additional high affinity over SSTR1 in GH release or over SSTR3 and SSTR5 in cell growth, apoptosis and immunoregulatory functions. In addition, it is suggested that the desensitisation resistance mechanisms to octreotide and lanreotide are less likely to be observed in prolonged SSTR inhibition with pasireotide.

Owing to the promising results obtained in preclinical and clinical studies including patients with acromegaly and Cushing's syndrome, investigation on NETs was initiated. A phase I clinical trial was conducted in 42 patients with well-differentiated GEP–NETs refractory to previous SA (56). The study aimed to analyse the pharmacokinetic and pharmacodynamic properties and tolerability of increasing doses of pasireotide LAR (20, 40 and 60 mg every 28 days) administered during 3 months. Fifteen patients presented grade 3/4 adverse events (diabetes mellitus and flushing were the most frequent; $n=3$ each). Steady-state levels were achieved before the third injection in the three groups. Efficacy was not assessed in this trial, but a contemporary phase II open-label trial analysed the symptom control rate during 15 consecutive days in 45 patients with functioning NETs and symptoms refractory to octreotide LAR (57). Patients without a complete or PR (partial/complete symptom relief and not more than 10% increase in biochemical parameters – 5HIAA and CgA) received increasing doses of pasireotide until 1200 μg/12 h. Twelve (27%) patients responded to treatment with pasireotide at a dose ranging from 600 to 900 μg bid. Tumour response based on RECIST did not identify any complete or PR, but 13 patients (57%) presented SD at the last radiological assessment (Table 2). The most frequent adverse events were nausea, abdominal pain, weight loss and hyperglycaemia occurring in more than 15% of patients. The blood glucose level was analysed in 25 patients, and the worse glucose control was observed in patients with diabetes mellitus or hyperglycaemia at baseline. Indeed, hyperglycaemia was transitory with treatment adjustments. An ongoing trial to optimise the treatment with pasireotide in NETs aims to determine the maximum tolerated dose over 60 mg monthly (Clinical Trial Number NCT01364415).

Telotristat etiprate (LX 1606)

A novel target in carcinoid tumours is the inhibition of the tryptophan hydroxylase (TPH) activity, which is involved in serotonin (5HT) release, responsible for the carcinoid syndrome symptoms. The expression of TPH is restricted to the intestinal and pancreatic enterochromaffin cells, β cells of the islets of Langerhans, mononuclear leukocytes, mast cells, pinealocytes and raphe neurons. Different genes encode the two different TPH isoforms, TPH1 and TPH2, located in enterochromaffin cells and CNS respectively (58).

Telotristat etiprate is an oral peripheral TPH inhibitor that has been developed to offer an additional treatment to patients suffering from carcinoid symptoms derived from hyperserotonaemia. From a phase I clinical trial, safety results were observed with a dose of 500 mg three times a day and the most common adverse events were mild to moderate nausea, diarrhoea and transaminase elevations. The development continues after the initial promising efficacy results in symptom relief (28% of patients with reduction in bowel movement) and biochemical response in u5HIAA observed in 56% of 23 patients included in a placebo-controlled escalating dose trial (59). An ongoing phase III trial is currently comparing

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the efficacy and safety of two different telotristat doses (250 and 500 mg tid) and placebo in patients with refractory carcinoid syndrome to SSAs (NCT01677910).

Chimeric somatostatin molecules

Somatostatin and dopamine are key molecules in neurotransmission and organ function regulation and their receptors share 30% of their gene sequence. Dopamine binds to a G protein-coupled DR and, preferentially, acts through the adenylate cyclase pathway (60). Two subfamilies of DRs have been described: D1-like receptors (D1 and D5) that activate adenylate cyclase signalling and D2-like receptors (D2, D3 and D4) that inhibit adenylate cyclase activity. Recent findings have identified the coexpression of both receptors in cell lines of endocrine tumours and suggested the possible dimerisation between SSTRs and DRs, offering a new target for research (61). In preclinical and clinical trials, chimeric molecules inhibiting SSTR2 and D2, such as BIM-23A758 or BIM-23A760, have been studied in other endocrine tumours (62). Although there is a strong basis for activity in GEP–NETs, investigation on BIM-23A760 has not progressed. However, further research in the development of new chimeric compounds is going on (7).

Immunotherapy

Currently, immunotherapy is representing a cornerstone in treatment strategies for different tumours as different checkpoints in immune system regulation are sensitive to targeted agents with antitumoural effect. Owing to the broad effective role of IFN in NETs, initial development of novel immune agents is being investigated, mainly against the inhibitory signals, such as CTLA4 and PD1. As there is no clear biomarker to predict the activity of novel immune agents, it is difficult to select the right NET patients in advance. There is a need to improve the profile of expression of novel targets such as PD1 (PDCD1) or its ligand throughout the different locations of primary NETs. To our knowledge, there is no active trial with these novel approaches specifically oriented to NETs. However, there are exclusive NET cohorts included in phase I trials, as well as PD1/PDL1 (CD274) expression analyses, demonstrating the great interest on this treatment strategy in NETs.

Conclusions

Biotherapy agents such as SSAs and IFN remain as the cornerstone of the systemic treatment of disseminated NETs despite the appearance of novel target agents such as sunitinib and everolimus. The role of biotherapy in symptom control and tumour growth has not been replaced till date.

In the absence of direct comparisons, biotherapy, mainly SSAs, should be considered as the initial approach for the systemic treatment of low- and intermediate-grade NETs regardless of the origin of the primary tumour site. Despite the lack of data on the baseline patients' characteristics, tumour grade and tumour response from early clinical trials, the disease control rate is similar in both treatments and midgut carcinoids seem to be more sensitive to the IFNα activity. However, the greater tolerability profile obtained with SSAs may help in treatment decision and leave IFN therapy as a valuable effective salvage therapy.

Initial results from retrospective data and clinical trials support the combination of SSAs with targeted therapies showing an acceptable tolerability profile and potential synergistic activity, taking into consideration the tumour proliferative index, the primary tumour location and the metastatic involvement. Chemotherapy based on platin combinations is significantly active in high-grade NETs and temozolomide, mainly combined with capecitabine, has emerged as an active strategy for patients with moderate and high (selected population)-grade NETs with a potential predictive biomarker (MGMT).

Recently, the β-emitting radionuclides ^{90}Y and ^{177}Lu conjugated to peptide receptor radiotargeted treatment (PRRT) are arising as effective treatments in GEP–NETs with promising results in non-randomised trials, achieving symptomatic relief and an ORR in up to 30% of patients and SD in 40–70% of patients (7). Currently, the first phase III clinical trial with PRRT in patients with progressive midgut NETs is going on (NCT 0178239). Patients are randomised to ^{177}Lu-DOTA0-Tyr3-octreotate or octreotide LAR 60 mg. However, there are no randomised face-to-face trials with PRRT, SSAs, IFN and chemotherapy to determine the specific role of each one in the treatment sequence. Furthermore, the efficacy of a combination strategy has not been defined.

Debulking surgery, when feasible, for the reduction in tumour burden, optimisation of symptom relief and prevention of obstructive complications is also recommended in advanced disease, as a complementary treatment modality. If palliative surgery is not possible in liver metastatic disease, which is mainly recommended in selected well-differentiated NETs, embolisation or chemoembolisation and radiofrequency ablation are also effective therapeutic options for local tumour control.

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Systemic therapy should be continued to control disease progression.

Altogether, the algorithm for the management of NETs is progressively getting more complex and better selection of patients from a clinical and molecular perspective is needed, including the introduction of reliable biomarkers, the comprehension of primary and acquired resistances to drugs or the definition of combined or sequential treatment regimens. Moreover, considering that the median survival of patients harbouring NETs is longer than the majority of solid tumours in advanced stages, it is really important to offer the best treatment sequence with all the active agents to our individual patients.

Finally, despite the certain activity and survival benefit demonstrated by biotherapy in NETs, several questions remain unresolved, such as the place that SSAs and IFN should take in the optimal sequencing to treat our patients, the best novel targeted agent that is more likely to have synergy with SSAs and IFN in NETs, the role for SSAs in the adjuvant setting of high relapse risk totally resected NETs, etc. Further investigation directed to maximise the selection of patients for the best management at any point of the disease should improve their global approach.

Declaration of interest

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

Funding

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

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Received 3 May 2014
Revised version received 10 August 2014
Accepted 14 August 2014

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