REVIEW ARTICLE

Peptide receptor therapies in neuroendocrine tumors

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ABSTRACT. Neuroendocrine tumors (NETs) are relatively rare tumors, mainly originating from the digestive system, able to produce bioactive amines and hormones. NETs tend to be slow growing and are often diagnosed when metastatic. The localization of a NETs and the assessment of the extent of disease are crucial for management. Commonly used diagnostic techniques include morphological imaging (ultrasound, computerized tomography, magnetic resonance), and functional imaging (somatostatin receptor scintigraphy, positron emission tomography techniques). Treatment is multidisciplinary and should be individualized according to the tumor type, burden, and symptoms. Therapeutic tools include surgery, interventional radiology, and medical treatments such as somatostatin analogues, interferon, chemotherapy, new targeted drugs and peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogues. NETs usually over-express somatostatin receptors, thus enabling the therapeutic use of somatostatin analogues, one of the basic tools, able to reduce signs and symptoms of hormone hypersecretion, improve quality of life, and slow tumor growth. PRRT with somatostatin analogues ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE has been explored in NETs for more than a decade. Present knowledge and clinical studies indicate that it is possible to deliver high-absorbed doses to tumors expressing sst_2 receptors, with partial and complete objective responses in up to 30% of patients. Side effects, involving the kidney and the bone marrow, are mild if adequate renal protection is used. Moreover, a consistent survival benefit is reported. As NETs may also express cholecystokinin 2, bombesin, neuropeptide Y or vasoactive intestinal peptide receptors even simultaneously, the potential availability and biological stability of radio-analogues will improve the multireceptor targeting of NETs.
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INTRODUCTION

Neuroendocrine tumors (NETs) are relatively rare tumors originating from dispersed neuroendocrine cells, distributed almost ubiquitously in the body. The term "neuroendocrine" relates to a peculiar characteristic or phenotype of these cells, namely the ability to synthesize, store, and secrete neuro-hormones, neuro-transmitters or neuromodulators (1, 2). Excluding small-cell lung carcinomas, a particularly aggressive tumor deserving separate considerations, the most frequent NETs occur in the digestive tract (66%), followed by the rest of the respiratory tract (31%) (3).

NETs tend to be slow growing (although there are aggressive types). They may present with symptoms related to the inappropriate peptide and neuro-hormone hypersecretion or, especially the non-functioning ones, which are the majority, with symptoms related to their mass effect. Even in functioning tumors, despite the presence of a distinct clinical syndrome, symptoms are frequently unrecognized, and diagnosis is often delayed to the metastatic phase, usually involving the liver (4).

The localization of a NET and the assessment of the extent of disease are crucial for management. Nowadays, commonly used diagnostic techniques include morphological imaging with transabdominal ultrasound, computerized tomography (CT), magnetic resonance (MRI), or endoscopic ultrasound, and functional imaging with widely available techniques like ^{111}In-octreotide (OctreoScan) or, more recently, somatostatin receptor positron emission tomography (PET) with ^{68}Ga-octreotide, as well as other experimental methods available only in few centres, such as ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide scintigraphy, or PET with ^{18}F-levo-DOPA, ^{11}C-5-hydroxytryptophan, or ^{86}Y-DOTATOC. No technique is the gold standard, and specific sequences of exams might be needed for each tumor type. Despite all efforts, a consistent number of NETs (up to 50%) remains with an unknown primary site (5-10).

Treatment of NETs is multidisciplinary and should be individualized based on the tumor type and burden, as well as symptoms. The therapeutic tools in NETs include surgery, interventional radiology, and medical treatments such as somatostatin analogues, interferon, chemotherapy, new targeted drugs and peptide receptor radionuclide therapy (PRRT) (4, 11, 12).

Surgery is fundamental in many phases, from the eradication of the primary, to the debulking of metastatic lesions, in view of other therapies, in order to control debilitating symptoms due to hormone overproduction or with a pure palliative intent (13, 14). The rationale of interventional radiology techniques in NETs relies in their common spread to the liver. Liver metastases from NETs are typically hypervascular and (chemo)embolization of the hepatic artery, performed mechanically by micro-

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spheres or also chemically with cytotoxic agents, can lead to significant necrosis (15). Recently, radioembolization of liver metastases with ^{90}Y-labelled microspheres has been tested in several clinical trials with excellent preliminary results (12). Other techniques still to be validated in NETs include "umbrella" radiofrequency ablation and the newest high-intensity focused ultrasound ablation (16, 17).

Medical therapy is aimed at treating symptoms and/or reducing tumor growth. Traditional chemotherapy has little place in well-differentiated NETs, since most of them are slow-growing tumors. Streptozotocin-based schemes in pancreatic tumors yielded significant objective responses, but none of the schemes used in "midgut carcinoids" showed any activity (18). Usually, protocols based on platinum derivatives and etoposide are considered in poorly differentiated and/or rapidly progressive NETs, but generally the choice of the particular regimen is based on the site of the primary and the histopathological differentiation.

One of the basic tools for NETs is somatostatin analogue biotherapy, combined or not with interferon. Somatostatin analogues are generally well tolerated and long-acting formulations are used successfully to control tumor hypersecretion and symptoms in up to 70% of patients, although tachyphylaxis frequently may occur (19, 20). Antiproliferative activity is scarce, with objective responses encountered in <10% of patients, while stabilization of disease is achieved in more than 60% (21). Interferons, and particularly α-interferon, have been used in the management of NETs, with therapeutic effects similar to those of somatostatin analogues, although the onset is delayed, but with more pronounced side effects. Presently, the combined use of α-interferon and somatostatin analogues as first-line therapy is not justified by data in the literature, while it could be indicated after progression to a single agent (19, 22).

Nowadays, new molecular drugs, targeting small cellular proteins or messengers involved in proliferation, are being experimented in phase II and III studies. The peculiar growth characteristics of NETs make them attractive targets for molecular-targeted therapies, since the longer period needed to progress allows drugs hitting the stromal or subcellular targets to demonstrate activity. The scenario is particularly ebullient, with many pharmaceuticals reaching the clinical phase. The most efficient and studied are vascular endothelial growth factor and mTOR inhibitors (23).

PEPTIDE RECEPTOR THERAPIES

Neuroendocrine cells are typically regulated by numerous hormones, acting *via* specific receptors on the membrane surface. These receptors are usually 7-transmembrane-domain G-protein-coupled receptors. The presence of a suitable density of internalising receptors on the cell surface of NETs poses the basis for a peptide receptor-targeted therapy. The most exploited and known ligand-receptor system in clinical practice, including nuclear medicine, is somatostatin. Agonists binding to somatostatin receptors are internalized into endosomes and activate post-receptor mechanisms, such as adenyl cyclase, phospholipase and ion channels, that are responsible for the pharmacological effect. The receptor is either recycled on the membrane surface or entrapped into lysosomes for degradation. This retention into the lysosomes allows a radionuclide-based peptide diagnosis and/or therapy, depending on the radionuclide used (24).

Tumors over-expressing somatostatin receptors, and candidates for radionuclide therapy, typically include pituitary adenomas, gastrointestinal and pancreatic endocrine carcinomas (the so-called GEP tumors), bronchial and thymic neuroendocrine tumors, paragangliomas, pheochromocytomas, small cell lung cancers, medullary thyroid carcinomas, breast cancers, and malignant lymphomas. Somatostatin receptors are expressed in a tissue- and subtype-selective manner in both normal and cancerous cells. Most of the above tumors express multiple receptor subtypes simultaneously, subtype 2 being the subtype most frequently detected. The presence of somatostatin receptors enables the treatment of tumor hypersecretion and of primary and metastatic lesion growth by somatostatin and its analogues, owing to post-receptor signaling, triggered by the receptor-ligand internalization (25).

Somatostatin analogues

All 5 somatostatin receptor subtypes (sst) bind with high affinity native somatostatin (both 14- and 28-amino acid isoforms). Somatostatin has an extremely short plasma half-life (about 2 min) and cannot be used for clinical purposes. Somatostatin-28 was first labelled with radioiodine (^{123}I), showing *in vivo* the rapid cleavage and metabolism that poorly allowed visualizing and therefore treating tumors (26). In the same years (beginning of 1980s), the octapeptide analogue octreotide was synthesized. Presently, octreotide, together with lanreotide, is the analogue approved for therapeutic clinical use. Both these analogues are mainly sst_2 preferring agents, showing therefore high affinity for sst_2 receptor, moderately high affinity for sst_5 and intermediate affinity for sst_3 (27).

"Cold" somatostatin analogues in clinical use

To date, the main clinical use of octreotide or lanreotide is limited to the symptomatic control of hypersecretory syndromes. Nevertheless, it has been used in various trials with the aim of testing its antiproliferative efficacy (28). In NETs of various origin the use of octreotide (0.5-1 mg tid) yielded symptomatic and biochemical responses in 73% and 77% of patients, respectively, with only 3% objective responses in carcinoids, in the evaluation of the Italian multicentre trial (29). The use of ultra high-dose lanreotide (up to 15 mg/day) seemed to give slightly higher tumor responses as well as biochemical and symptomatic responses (>6%) (30, 31).

Somatostatin radio-analogues for peptide-receptor radionuclide therapy

^{111}In-labelled octreotide was approved by the Food and Drug Administration (FDA) in 1994 as a diagnostic agent for scintigraphy of patients with NETs. Once octreotide was radiolabeled for diagnostic imaging in order to localize tumor lesions over-expressing somatostatin receptors (32), the next logical step was to develop PRRT.

The theoretical basis of such therapy is principally to convey radioactivity inside the tumor cell, owing to the internalization of the somatostatin receptor and radiolabeled analogue complex. The first attempts to perform PRRT with radiolabeled octreotide began in the 1990s in a multicentre trial using high activities of ^{111}In-octreotide. The results obtained, in terms of clinical benefit and overall responses are due to the Auger and conversion electrons emitted by Indium-111, decaying in close proximity to the cell nucleus, once that peptide/receptor complex has been internalized. Despite these premises, partial remissions were exceptional (33).

Higher-energy and longer-range emitters, such as pure β emitter Yttrium-90 (E_{max} 2.27 MeV, R_{max} 11 mm, $T_{1/2}$ 64 h) seemed more suitable for therapeutic purposes. Therefore a new analogue, Tyr^3-octreotide, with a similar pattern of affinity for somatostatin receptors, was developed for its high hydrophilicity, simple labeling with ^{111}In and ^{90}Y, and tight binding to the macrocyclic chelator DOTA (1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetraacetic acid), to form ^{90}Y-[DOTA]^0-Tyr^3-octreotide or ^{90}Y-DOTA-TOC (34). Recently, a newer analogue, named octreotate (Tyr^3,Thr^8-octreotide) with 6- to 9-fold higher affinity for sst_2 was synthesized. The chelated analogue [DOTA]^0-Tyr^3-octreotate or DOTATATE can be labeled with the β-γ emitter Lutetium-177 ($E_{\beta max}$ 0.49 MeV, $R_{\beta max}$ 2 mm, $T_{1/2}$ 6.7 days) and has been experimented in clinical studies. Theoretically, Auger-electron emitters represent an attractive alternative to β-particle emitters for cancer therapy if they can be placed intracellularly, especially in close proximity to (or within) the nuclear DNA. Incorporation of Auger-electron emitters into the DNA is a particularly efficient source of irradiation, capable of inducing cell death with virtually no damage to the surrounding cells. Experience in this field comes from a radiolabeled thymidine analogue, IUdR, which represents the most extensively explored radiobiologic model for cancer therapy with Auger-electron emitters. Upon incorporation of iodine-125 into DNA, the disintegration of this Auger-electron-emitting isotope has a relative biologic effectiveness 7- to 8-fold greater than equivalent amounts of β or γ emission. There is now sufficient evidence to show that generally, the intra-nuclear localization and specifically intercalation or at least the proximity of Auger-electron emitters to the double-stranded nuclear DNA determine their cytotoxicity. Coming to somatostatin analogues, it has been extensively discussed whether ^{111}In-octreotide locates targets placed inside the cell nucleus. Studies in literature are scant and contradictory, nonetheless nuclear uptake is likely to be scarce, and this seems to be the explanation of such poor results in clinical trials. Since the beginning of the new century, PRRT has been performed only with β-emitters (35).

Several new peptides have been introduced in nuclear medicine for therapeutic and diagnostic purposes, such as new sst_2 agonists DOTA-TATE, DOTA-NOC, and DOTABOC-ATE (where NOC is [1-Nal^3]-octreotide; and BOC-ATE is [BzThi^3,Thr^8]-octreotide). Each one can be labeled with either therapeutic radiometals, such as Yttrium-90 or Lutetium-177, or with positron-emitters, such as Gallium-68, for PET-receptor imaging, thus giving rise to different radiopeptides as to their biological and clinical properties, and many of them are already used in diagnostic and therapeutic trials (36).

Other potential receptors and (radio)peptides for therapy

Somatostatin receptor system represents an actual treatment pathway and a model for future tumor therapies. Many ligand-receptor systems have been discovered in different human tissues, such as dopamine, bombesin, cholecystokinin, vasoactive intestinal peptide, substance-P and others, which could represent adjunctive targets for "cold" and radiolabelled analogue therapy (Table 1). Regarding dopamine receptors, the first intuitions of their possible presence in NETs started from the observation that ^{123}I-epidepride, a D2 dopamine receptor antagonist, could be used in pituitary imaging in substitution of an iodinated benzamide, ^{123}I-IBZM, known also to accumulate in melanomas. ^{123}I-epidepride was then shown to accumulate in human melanomas, and dopamine D2 receptors were therein demonstrated, also by means of other techniques (43, 44). Subsequently D2 receptors were demonstrated in NETs as well, such as those associated with ectopic ACTH syndrome. Furthermore, cabergoline, a new D2-receptor synthetic analogue demonstrated efficacy in controlling cortisol excesses in some patients (45). Cabergoline also seems to increase the efficacy of somatostatin analogs in controlling ectopic Cushing syndrome (46).

Moreover, recent observations have shown that internalization of human somatostatin receptors could be determined by functional homo- and heterodimerization with somatostatin receptors or other G-protein-coupled receptors, such as dopamine D2 receptor, with resulting properties that differ completely from those of the individual receptors as to ligand-binding affinity, signaling, agonist-induced regulation and internalization. The effects of newer analogues, such as sst_2/sst_5, sst_2/D2 and sst_2,sst_5/D2 (dopastatin) bi- or tri-hybrid chimerical analogues have been explored *in vitro* in primary cultures of GH-secreting pituitary adenomas partially responding to conventional somatostatin analogues, and are being experimented also in NETs (47-49).

For the moment, the lack of selectivity for basal ganglia and tumor shown by D2 receptor ligands and possibly by chimeras, makes them unsuitable for designing a radionuclide therapy.

Presently, somatostatin analogue binding 4 out of 5 ssts, the so-called pan-agonist SOM-230 (pasireotide), which binds with high affinity $sst_{1,2,3}$ and sst_5, is being experimented in clinical trials for the therapeutic control of NETs, but, given the wide systemic expression of the receptor subtypes other than sst_2, pan-agonists are far from being used in radionuclide therapy (50).

Finally, the demonstration, in animal models, of a far superior tumor targeting by non-internalising somatostatin receptor antagonists is revolutionizing the paradigm of the internalization of the receptor-ligand complex as the basis for PRRT (51).

PRRT with radiolabeled somatostatin analogues

Nowadays, tumor candidates for PRRT with radiolabeled somatostatin analogues are basically sst_2 expressing

Table 1 - *Ligands used in clinical practice for diagnosis of neuroendocrine tumors and their relative therapeutic counterparts (ligands in development are shown in italics) (37-42).*

Target	Ligand	Diagnostic radiopharmaceutical	Therapeutic radiopharmaceutical
Somatostatin receptors	Somatostatin analogues	^{111}In-DTPA-D-Phe^{1}-Octreotide (-Pentetreotide)	^{111}In-Pentetreotide
		^{111}In-DOTA-Lanreotide	^{90}Y-DOTA-Lanreotide
		^{111}In-DOTA-Tyr^{3}-Octreotide (-DOTATOC)	^{90}Y-DOTATOC
		^{99m}Tc-Depreotide (P829)	n.a.
		^{99m}Tc-EDDA/HYNIC-Tyr^{3}-Octreotide	n.a.
		^{99m}Tc-Vapreotide (RC160)	n.a.
		^{111}In-DOTA-Tyr^{3},Thr^{8}-Octreotide (-DOTATATE)	^{177}Lu-DOTATATE
		^{68}Ga-DOTA-1-Nal^{3}-octreotide (-DOTANOC)	^{90}Y-DOTANOC
		^{68}Ga-DOTATOC	^{90}Y-DOTATOC
		^{64}Cu-TETA-octreotide	n.a.
CCK2-gastrin receptors	CCK-gastrin analogues	^{111}In-DTPA-D-Asp^{26}-Nle^{29},^{31}-CCK	n.a.
		^{111}In-DTPA-d-Glu^{1}-minigastrin	^{90}Y-DTPA-D-Glu^{1}-minigastrin
VIP receptors	VIP analogues	^{123}I-VIP	n.a.
		^{99m}Tc-TP3654	n.a.
GRP-receptors	Bombesin analogues	^{99m}Tc-RP527	n.a.
		^{111}In-DTPA-Pro^{1}Tyr^{4}-bombesin	n.a.
		^{99m}Tc-Demobesin	n.a.
		^{177}Lu-AMBA (or BBN8)	^{177}Lu-AMBA
NK-1 receptors	Substance P analogues	^{111}In-DTPA-Substance P	^{90}Y-DOTAGA-substance P
GLP-1 receptors	GLP-1 analogues	*^{111}In exendin-4*	n.a.
NT-1 receptors	Neurotensin analogues	*^{99m}Tc-NT-XI*	n.a.
Oxytocin receptors	Oxytocin analogues	*^{111}In-DOTA-Lys^{8}-vasotocin*	n.a.
Catecholamine transport and storage	Catecholamine	^{18}F-DOPA	n.a.
		^{123}I-mIBG	^{131}I-mIBG
		^{18}F-dopamine	n.a.
Serotonin receptor	Serotonin	^{11}C-5-HTP	n.a.
GLUT (glucose transporter)	Glucose	^{18}FDG	n.a.

CCK: cholecystokinin; VIP: vasoactive intestinal peptide; GRP: gastrin-releasing peptide; GLP: glucagon-like peptide; n.a.: not available.

NETs, mainly of the GEP and bronchial tract, but also pheochromocytomas, paragangliomas, medullary thyroid carcinomas, and, at least theoretically, any other tumor histotype known and documented as over-expressing membrane sst_2. Among the inclusion criteria, a high expression of functioning, namely internalising somatostatin receptors is critical for efficient therapy. Somatostatin receptor scintigraphy is presently the most accurate method to check for the presence of functioning somatostatin receptor over-expression. Immunohistochemistry for sst_2 can also be performed but, being a sort of photograph taken at the moment of bioptic sampling, the actual internalising capacity and the possible evolution in time of receptor density cannot be assessed. A correlation between immunohistochemical profile in NETs and the in vivo scintigraphy features has been explored in a recent study (52). However, larger cohorts of patients are warranted to draw conclusive results. Moreover, the receptor status in the remainder of tumor sites cannot obviously be assessed and cannot always be assumed to be homogeneous. Somatostatin receptor scintigraphy has indications in the localization, staging and follow up of a NET; however, the ability to select patients to be submitted to "cold" or radiolabeled somatostatin analogues is the most peculiar. When analysing a scan, it is important to exclude possible false positives, such as gallbladder, accessory spleens, recent surgical scars, and any other cause of granulomatous-lymphoid infiltrate that may mimic a tumor lesion. In addition, possible cases of false negatives must be excluded, particularly sub-centimetrical lesions under the resolution power of the method, recent chemotherapy, or dedifferentiated disease.

PRRT consists in the systemic administration of the radiopeptide, such as ^{90}Y-DOTATOC or ^{177}Lu-DOTATATE, the most used ones, divided in sequential cycles, administered 6-9 weeks apart, up to a cumulative activity that is calculated basing on renal irradiation.

PRRT efficacy

Before considering the clinical outcome of PRRT, the theoretical principles at the basis of the efficacy must be summoned up, namely the radiosensitivity and the radioactive concentration on tumor site. Actually, NETs are not particularly radiosensitive (53), and this is an intrinsic characteristic involving the growth pattern and the DNA repair capability. On the other hand, the radioactive concentration at the tumor site is crucial and can be modulated. In fact, the higher is the concentration of radioactivity in the tumor, the higher the probability of its shrinkage. In order to increase the amount of radioactivity at the target, and therefore the so-called target-to-back-

ground ratio, the kinetics characteristics of the radiopeptide used, its affinity for the receptor, and the receptor density on tumor cells, must be taken into account.

The pharmacokinetics profile of DOTATOC, and similarly of DOTATATE, is remarkably favorable, with a rapid plasma clearance after administration [less than 9%±5% of injected dose (i.d.) within the first hour to less than 0.9%±0.4% within 10-12 h after injection] and the renal excretion is relevant (73%±11% i.d. in urine after 24 h) (54).

The various available octreotide derivatives possess variable affinity profiles for sst_2, sst_3 and sst_5. Peptides such as DOTATOC and even more DOTATATE and DOTANOC possess a high affinity for sst_2, the most widely expressed receptor in NETs (11, 1.5, and 3.3 IC_{50} nM, respectively). Analogues showing high affinity for sst_3 and sst_5, such as DOTANOC (26 and 10 IC_{50} nM for sst_3 and sst_5, respectively), can also be exploited in tumors, such as thymic tumors or follicular thyroid carcinomas, presenting a relatively higher expression of these subtypes (39).

Finally, the receptor density on tumor *vs* normal organs must be considered as well. The higher the density, the greater the amount of radiopeptide that may be conveyed inside the tumor cells. In clinical practice, the density is evaluated by means of receptor scintigraphy, according to a visual scale, named the "Rotterdam scale", where tumors candidate to PRRT are those with an uptake on planar images at least equal to the one of the normal liver (grade 1), higher than that (grade 2) or higher than the one of kidneys and spleen, the "hottest" organs at ^{111}In-octreotide scintigraphy (grade 3). Tumor remission, in fact, is positively correlated with a high uptake at receptor scintigraphy. Nevertheless, tumor radiation dose does not only depend directly on the administered activity and the uptake *vs* time, but also on the tumor mass. Smaller masses have higher chances of mass reduction, owing to a higher absorbed dose in the tumor. This is confirmed by clinical data regarding the characteristics of response: patients with limited number of liver metastases respond to PRRT, whilst patients with a high tumor load do not (55). Considering PRRT with the two most exploited radiopeptides, ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE, mathematical models showed that ^{177}Lu is better in small tumors (optimal diameter 2 mm), whilst ^{90}Y is in larger ones (optimal diameter 34 mm). Very small masses, in fact, are likely not to absorb all the β-energy released in the tumor cells by ^{90}Y, while larger tumors will suffer from the lack of uniformity of activity distribution of ^{177}Lu. Finally, differences in dose-rate must be taken into account: the longer physical half-life of ^{177}Lu means a longer period needed to deliver the same radiation dose as ^{90}Y. This may allow more time for tumor re-population. Therefore, a combination therapy with ^{90}Y and ^{177}Lu, either simultaneously or in distinct settings, has been suggested to overcome the difficulties of real clinical situation of different sized lesions (56).

PRRT safety

Due to their marked radiosensitivity, the kidneys are the critical organs in PRRT, particularly after ^{90}Y-DOTATOC administration. Proximal tubular reabsorption of the radiopeptide and the subsequent retention in the interstitium results in renal irradiation. Nephrotoxicity is accelerated by risk factors, such as pre-existing hypertension or diabetes. Given the high kidney retention of radiopeptides, positively charged molecules, such as L-lysine and/or L-arginine, are used to competitively inhibit the proximal tubular re-absorption of the radiopeptide. This leads to a reduction in the renal irradiation dose ranging from 9 to 53% (57-59). Renal doses are further reduced up to 39% by prolonging infusion over 10 h and up to 65% by prolonging it over two days after radiopeptide administration, thus more extensively covering the elimination phase through the kidneys (8, 60). Despite kidney protection, renal function loss may become clinically evident years after PRRT. A median decline in creatinine clearance of 7.3% per year was reported in patients treated with ^{90}Y-DOTATOC and of 3.8% per yr in patients treated with ^{177}Lu-DOTATATE. Cumulative and per-cycle renal absorbed dose, age, hypertension, and diabetes are considered as contributing factors to the decline of renal function after PRRT (61).

Kidney radiation toxicity is typically evident several months after irradiation, due to the slow repair characteristics of renal cell. According to studies on renal toxicity derived from external radiotherapy (those referred to by the nuclear medicine community, up to a few years ago), the accepted renal tolerated dose is in the range of 23-25 Gy. As stated by the National Council on Radiation Protection and Measurements (NCRPM) in fact, a dose of 23 Gy to the kidneys causes detrimental deterministic effects in 5% of patients within 5 yr) (62, 63).

Nevertheless, clinical experience and dosimetric studies clearly indicate that this renal dose threshold does not accurately correlate with the renal toxicity observed in patients undergoing PRRT (64).

PRRT is a form of continuous radiation delivery with a decreasing dose-rate with time. The irradiation produces both lethal and sub-lethal damage, which can be repaired during the irradiation itself, but the differential between creating new damage and the repairing depends on the specific dose-rate at any particular time and on the repair capability ($T_{½rep}$) of the tissue. Low-dose rates, as in PRRT, will spare normal tissues more than the tumor and this may allow benefits as in fractionation in external radiotherapy (65).

The linear quadratic model mathematically interprets this differential sparing and the biological effective dose (BED) concept is used to quantify the biological effects induced by different patterns of radiation delivery. This model has been recently revised for radionuclide therapy and has been applied in particular to PRRT with the intent of increasing the dose-response correlation (66). Focusing on the kidney concern, the BED has proven to be a reliable predictor of renal toxicity, helpful in the implementation of individual treatment planning (64). However, BED is a relatively young concept applied to nuclear medicine and has still to be fully validated with a wider series of data.

The main radiobiological parameter required in such assessment is the tissue α/β ratio, which gives an indication of the sensitivity of a tumor or normal tissue cell to the effect of dose-rate (and/or fractionation), and is generally higher for tumors (5-25 Gy) than for late-responding normal tissues (2-5 Gy), such as the kidneys.

Renal toxicity is not the only parameter to be considered. Although it appears not to be the principal dose-limiting factor, bone marrow involvement must be taken into account as well. Usually, PRRT is well tolerated and severe, grade 3 or 4, hematological toxicity does not account for >13% of patients treated with ^{90}Y-DOTATOC and 2-3% of those treated with ^{177}Lu-DOTATATE (Table 2). The possibility of a mild, but progressive impoverishment in bone marrow reserves has to be considered in repeated cycles, particularly after ^{90}Y-DOTATOC, while recovery appears to be virtually complete after ^{177}Lu-DOTATATE. In addition, the possibility of myelodysplastic syndrome (MDS) or overt leukemia in patients receiving high bone marrow doses, especially in those previously treated with alkylating agents, must be considered (60, 67). Fertility can be temporarily impaired in males, due to damage to Sertoli cells, as testified by a drop in inhibin-B and a contemporary increase in FSH. Usually, fertility is restored within 12-18 months from the end of therapy (55). Finally, it must be considered that treating functioning NETs with PRRT may result in acute cell rupture and hence exacerbation of clinical syndromes, such as hypoglycemia, carcinoid or Zollinger-Ellison syndromes, sometimes to severe degrees, requiring further hospitalization (68).

PRRT clinical results

Several clinical phase I-II trials indicated that PRRT with radiolabeled somatostatin analogues is amongst the promising newly developed targeted tools in NETs, with registered objective responses in up to 30% of patients (Table 2) (67).

Initial studies were performed with the administration of high doses of the radiopeptide used in diagnostics, ^{111}In-octreotide. The rationale is based on the emission of Auger and conversion electrons by Indium-111, decaying in close proximity to the cell nucleus after the internalization of the peptide/receptor complex. Objective responses were rare due to the short range of the emission (0.0025 μm) of the particles. Amongst 40 patients treated with cumulative doses of 20 to 160 GBq, 1 partial remission, 6 minor remissions, and 14 stabilizations of disease were reported. Mild hematological toxicity was observed, but 3 cases of MDS or leukemia occurred in the patients treated with high activities (>100 GBq) and high estimated bone marrow doses (about 3 Gy). In another study in 27 patients with GEP NETs, partial responses occurred in 2 of 26 patients with measurable disease. Renal insufficiency was reported in one patient, although possibly not treatment-related (33, 69).

The radiopeptide that has been most extensively studied is ^{90}Y-DOTATOC. All the published results derive from phase I-II trials, and were inhomogeneous as to patient selection, inclusion criteria, treatment schemes and dosages (cumulative activities ranged from 2 to 32 GBq). Therefore, an inter-study comparison is virtually impossible. Nevertheless, despite differences in clinical phase I-II protocols from various centres, complete and partial remissions were registered in 10 to 30% of patients, a rate undoubtedly higher than that obtained with ^{111}In-octreotide. In a first report, 29 patients were treated with a dose-escalating scheme consisting in 4 or more cycles of ^{90}Y-DOTATOC with cumulative activities of 6.12±1.35 GBq/m^2. Twenty of these patients showed disease stabilization, 2 had partial remission, 4 minor remission, and 3 progressed (70). In a subsequent study, 39 patients were treated with 4 equal intravenous injections, for a total of 7.4 GBq/m^2 of ^{90}Y-DOTATOC. The objective response rate was 23%, with complete remission in 2 patients, partial remission in 7, and stabilization in 27. Pancreatic NETs (13 patients) showed a higher objective response rate (38%). A significant reduction of clinical symptoms was recorded (71). Toxicity was generally mild and involved the kidney and the bone marrow. However, renal insufficiency was reported in 5 patients not receiving renal protection during the therapy, while severe hematological toxicity occurred in those patients treated with high cumulative activities.

Dosimetric and dose escalating studies with ^{90}Y-DOTATOC, with and without renal protection with amino acids, showing no major acute reactions were observed up to an administered dose of 5.55 GBq per cycle (72). Reversible grade 3 haematological toxicity was found in 43% of patients injected with 5.18 GBq, which was defined as the maximum tolerated dose per cycle. None of the patients developed acute or delayed kidney toxicity, although follow-up was short. Partial and complete responses were reported in 28% of 87 patients with NETs. In the multicenter phase 1 study, 60 patients received escalating doses up to 14.8 GBq/m^2 in 4 cycles or up to 9.3 GBq/m^2 in a single dose, without reaching

Table 2 - *Major features of safety and efficacy in peptide receptor radionuclide therapy with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE in the main published studies (modified from 67).*

	Efficacy		Safety			
	No. of patients	CR+PR (%)	No. of patients	Hematological grade 3 and 4 (%)	Renal insufficiency*	Other*
^{90}Y-DOTATOC						
Milan (72)	21	29	40	≤7	1 case reported	
Basel (71)	33	33	29	≤2	5 cases reported	
Rotterdam (73)	58	20	58	≤13	1 case reported	1 MDS
^{177}Lu-DOTATATE						
Rotterdam (55)	125	28	125	<2	1 case reported	1 MDS

*Renal failure and myelodysplastic syndrome (MDS) or overt leukemias have been reported by the various groups, mainly as personal communications; published data are still lacking and studies are ongoing. Therefore, an exact calculation of the incidence of these adverse events is not possible, although, especially in case of MDS, the incidence does not seem to be higher than in the normal oncological population and the estimate is frequently hampered by the occurrence of previous myelotoxic chemotherapies. CR: complete response; PR: partial response.

the maximum tolerated single dose. All patients received renal protection. Three patients had dose-limiting toxicity: 1 had liver toxicity, 1 had grade 4 thrombocytopenia, and 1 had MDS. Four of 54 patients (8%) treated with the maximum allowed dose had partial response, and 7 patients (13%) had minor responses. The median time to progression in the 44 patients showing stable disease, minor or partial response was 30 months (73).

Genuine phase II studies with ^{90}Y-DOTATOC are still lacking, but experiences in selected series of patients, mostly retrospective, are reported in the literature. A tentative categorization of the objective response according to the tumour type has been attempted in a metanalysis of results in GEP tumors. Pancreatic NETs proved to be the ones responding better to PRRT (67). Other limited experiences in medullary and follicular thyroid carcinomas, lympho-proliferative disorders, pheochromocytomas and paranganglioma are reported.

^{90}Y-DOTATOC (7.5-19.2 GBq in 2-8 cycles) has been administered in 21 patients affected by metastatic medullary thyroid carcinoma with positive OctreoScan, progressing after conventional treatments. Two patients (10%) obtained a complete response, as evaluated by CT, MRI and/or ultrasound, while a stabilization of disease was observed in 12 patients (57%); 7 patients (33%) did not respond to therapy. The duration of the response ranged between 3 and 40 months. Using biochemical parameters (calcitonin and carcinoembryonic antigen), a complete response was observed in 1 patient (5%), with partial response in 5 patients (24%) and stabilization in 3 (14%). Twelve patients had progression (57%). Complete responses were observed in patients with lower tumor burden and calcitonin values at the time of the enrolment. This retrospective analysis is consistent with the literature, regarding a low response rate in medullary thyroid cancers treated with ^{90}Y-DOTATOC. Nevertheless, patients with smaller tumors and higher uptake of the radiopeptide tended to respond better (74).

An interesting perspective of PRRT in lympho-proliferative disorders is opened by the presence of ssts in B-lymphocytes, but today no data are available regarding their use as targets for therapy. Sporadic observations are reported in literature, such as the case report of successful PRRT with ^{90}Y-DOTATOC in B-chronic lymphocytic leukemia in a patient affected by Binet A-chronic lymphocytic leukemia and advanced neuroendocrine Merkel carcinoma. The presence of somatostatin receptors both in normal and neoplastic B cells, and the usual drop of lymphocytes normally observed after ^{90}Y-DOTATOC, constitutes the basis for setting up PRRT specifically in B-cell lymphoma and leukemia (75).

As to the survival after ^{90}Y-DOTATOC, a phase I-II study on 58 patients with GEP NETs treated with 1.7-32.8 GBq reports a clinical benefit (including stabilization and minor response) in 57% (with true objective response in 20%), a median overall survival of 36.7 months (*vs* 12 months in the historic group treated with ^{111}In-octreotide), and a median progression-free survival of 29 months. Characteristically, patients stable at baseline had a better overall survival than had patients progressive at baseline, and the extent of disease at baseline was a predictive factor for survival (76).

The newer somatostatin analogue [DOTA^0,Tyr^3,Thr^8]-octreotide or DOTATATE has a 9-fold higher affinity for the sst_2 compared with [DOTA^0,Tyr^3]octreotide *in vitro*. Radiolabeling with the β/γ-emitter Lutetium-177 yielded tumor regressions and prolonged animal survival in a rat model (77, 78). In a preliminary report by the Rotterdam group, 35 patients with GEP NETs were treated with 3.7, 5.6, or 7.4 GBq of ^{177}Lu-DOTATATE, up to a final cumulative dose of 22.2-29.6 GBq, with complete and partial responses in 38%. No serious side effects were observed (79).

In a subsequent larger study, 131 patients with somatostatin receptor-positive tumors were treated with up to a cumulative dose of 22.2 to 29.6 GBq of ^{177}Lu-DOTATATE. One patient developed renal insufficiency, and another patient developed hepato-renal syndrome. Severe hematological toxicity occurred after <2% of the administrations. In the 125 evaluated patients, complete remission was observed in 3 patients (2%), partial remission in 32 (26%), minor response in 24 (19%), and stable disease in 44 patients (35%), while 22 patients (18%) progressed. Better responses were more frequent in case of a high uptake on baseline octreotide scintigraphy and in cases where a limited number of liver metastases were present, while progression was significantly more frequent in patients with a low performance score and extensive disease at enrolment. Median time to progression was more than 36 months, comparing favorably to chemotherapy. In addition, ^{177}Lu-DOTATATE significantly improved the global health/Quality of Life and various functions and symptom scales in patients with metastatic GEP tumors. The effect was more frequent in patients obtaining tumor regression, but, surprisingly, was observed also in progressing patients (55, 80).

A categorization of objective response showed once more that pancreatic tumors tended to respond better than other GEP tumors, although pancreatic gastrinomas tended to relapse in a shorter interval (median TTP 20 months *vs* >36 in the rest of GEP tumors) (55). In another study, traditionally poor responding tumors, such as bronchial and gastric neuroendocrine carcinomas, were included. Despite the limited number of patients studied, the observed objective response was comparable to the one observed in GEP NETs (81).

Recently, an evaluation of the enlarged series of 504 patients treated with ^{177}Lu-DOTATATE, 310 of which evaluated for efficacy, along with the confirmation of the occurrence of complete and partial remissions in 2 and 28% of cases, demonstrated a survival benefit of 40 to 72 months, compared to historical controls. Even with the limitations of such a comparison, the huge difference in survival is most likely to be caused by a real impact of PRRT (82).

In conclusion, PRRT with ^{90}Y-DOTATOC or ^{177}Lu-DOTATATE proved to be effective, with up to 30% objective responses, and reasonably safe up to 25-27 Gy to the kidneys, with an acceptable toxicity to kidneys and bone marrow. Nevertheless, some open questions remain, such as the most correct timing of PRRT. PRRT is a relatively young treatment, the majority of the results derive from phase I-II studies, and therefore the exact place of PRRT in the therapeutic work-up of NETs remains to be established. The first studies, in fact, were carried out in

relatively advanced phases of disease, while further trials demonstrated a higher efficacy of PRRT in earlier phases of disease. This is supported by numerous reasons, primarily radiobiological, since the smaller the tumor mass, the higher the dose, and also biological, since more advanced tumors bear many genetic mutations, such as p53, which make them less prone to respond to any treatment. Previous studies have indicated that the tumor load, especially in the liver, and the performance status would influence the outcome of PRRT. Therefore, early treatment rather than a "wait and see" approach could be advantageous. In addition, the type of disease has to be taken into account, as e.g. pancreatic NETs tend to respond better. Uniform pathology-oriented phase II trials are required to assess the potential of PRRT, as well as the best candidate to this treatment, from a clinico-pathological point of view.

Another open question is which is the optimal radio-peptide and, even before that, which of the two experimented is the optimal radionuclide. Theoretical considerations and animal studies showed the better suitability of Yttrim-90 for bigger lesions (optimal diameter 34 mm) and of Lutetium-177 for smaller lesions and micrometastases (optimal diameter 2 mm) (56). Nevertheless, the demonstration of high rates of objective responses with ^{177}Lu-DOTATATE in patients not selected for lesion size impairs these pre-clinical observations and beseeches further comparative studies between ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE. However, some considerations can be made in the meantime. The analysis of the residence times for DOTATATE and DOTATOC, calculated by means of the ^{177}Lu-labelled peptides, showed that residence times for DOTATATE are significantly longer in kidney and tumor (ratios DOTATATE: DOTATOC = 1.4 and 2.1, respectively), allowing higher tumor doses but also higher renal doses (83). Therefore, considering the higher tumor dose, ^{177}Lu appears more beneficial when labeling DOTATATE, while, in view of the higher renal dose, ^{90}Y appears more convenient to label DOTATOC. From dosimetric projections, we can infer that, for peptides such as DOTATATE, switching the radiolabel from ^{177}Lu to ^{90}Y can increase the doses by a factor 2 to 4 to the tumor, depending on the tumor size, but also to normal organs, kidneys in particular. Therefore, the benefit/risk balance remains to be established for each patient (Cremonesi M., EANM congress 2006, personal communication).

In conclusion, from a dosimetric point of view ^{177}Lu-DOTATATE appears handier than ^{90}Y-DOTATOC, as regards safety. However, ^{90}Y-DOTATOC is more powerful than ^{177}Lu-DOTATATE, as regards the tumor dose. The choice of the radiopeptide depends on the particular clinical scenario of the patient. Bigger lesions may benefit from ^{90}Y-DOTATOC while smaller ones from ^{177}Lu-DOTATATE. Especially when using ^{90}Y-DOTATOC, particular attention has to be paid to risk factors for renal toxicity, that should suggest caution (lower doses, hyperfractionation) or switching to ^{177}Lu-DOTATATE. In order to establish which treatment scheme and which radiolabeled somatostatin analogue or combination is optimal, a clinical randomized study comparing the two treatments is needed.

Future perspectives include studies addressed at exploring the effects of the combined use of PRRT with other drugs, such as radiosensitising chemotherapeutic agents like capecitabine, which showed some adjunctive antitumor activity without major side effects, or anti-angiogenetic drugs (84).

As GEP NETs may also express cholecystokinin 2, bombesin, neuropeptide Y, or vasoactive intestinal peptide receptors, even simultaneously, the potential availability and biological stability of radio-analogues of these peptides will in future improve the multireceptor targeting of the neuroendocrine cell (85).

CONCLUSIONS

Many different somatostatin receptor binding analogues have now been described, radiolabeled with ^{123}I, ^{111}In, ^{99m}Tc, ^{68}Ga or ^{18}F for diagnostic purposes. These proved to be an excellent tool for the clinical management of patients with NETs. Not only has diagnosis been eased with these radiopharmaceuticals together with radiological techniques but also useful information for staging and therapy decision making has been provided. When radiolabeled with β-emitting isotopes, such as ^{90}Y and ^{177}Lu, the same peptides have been successfully used for peptide-based radio-therapy (PPRT), with few serious adverse effects, important tumor responses and long progression-free survival rates. This field is rapidly growing and improving; new agonist and antagonist peptides have been described that can soon be tested in clinical trials.

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